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A DISCUSSION OF THE ETIOLOGY OF LEPROSY, WITH ESPECIAL REFERENCE TO THE POSSIBILITY OF THE TRANSFERENCE OF LEPROSY BY INSECTS, AND THE EXPERIMENTAL INOCULATION OF THREE MEN

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SIX TEXT FIGURES

INTRODUCTION

Although there can be no manner of doubt that leprosy is an infectious disease and that as such it can only be acquired from an antecedent case, nothing accurate is known concerning the method or methods by which the disease is transferred or of how or where lepra bacilli enter the body.

With regard to the portal of entry for the infection, practically all evidence obtainable indicates that the bacilli probably enter the body through the skin. Infection through the alimentary canal and through the genital organs has always been considered as highly improbable from the nature of the disease and its known epidemiological characteristics. On the other hand, a number of writers have claimed the respiratory tract as the common portal of entry. The lungs may be easily dismissed since they are rarely affected by leprosy and then at a late period of the disease; but nasal lesions are among the commonest lesions of leprosy, and some authors have found lesions and bacilli in the nose to be the most constant single symptom

¹ From the United States Army Medical Department Research Board.

in leprosy. Heiser found it in 799 out of 1,200 lepers. But most of Heiser's lepers were advanced cases. Solis and Wade examined 250 children above 3 years of age who developed leprosy at Culion, and in 60 per cent found lesions in the skin only. They failed to find a case with positive nasal smears in the absence of positive skin lesions. Other authors have reported finding bacilli in the nose rarely in early cases, and Neisser stated that he had never seen a single case of exclusive leprosy of the nose. The frequency of the first observable lesion on the face and the free communication with the lymph channels of the nose may readily account for the occurrence of early nasal infection. Most authorities are of the opinion that while in certain cases the nose may be a portal of entry, this cannot be accepted as the common method of transmission.

This leaves us only the skin as the common portal of entry. In many, perhaps the majority of cases, a sharply localized cutaneous lesion is the first demonstrable lesion of leprosy; and while it must be admitted that in any given case it is impossible to prove that these lesions are the first and only manifestations of leprosy, still this is a strong probability. Many such observations have been made of which the following may be quoted. Cognac and Mougeot⁽¹⁾ found in Cochin China, that of 2,437 cases, the first sign of leprosy appeared 550 times on the feet, 420 times on the hands, 321 times on the hands and feet, and 337 times on the face. Callender and Bitterman⁽²⁾ in a study of 259 lepers in the Philippines found that the first observed lesion of leprosy occurred on an exposed portion of the body in 95.5 per cent of the cases. The significance of this observation is reduced by the fact that in 78.2 per cent of the cases the first symptom observed was anæsthesia which indicates a nerve involment. Nevertheless, the early involment of the nerves by no means excludes the skin as the portal of entry, since the bacilli so entering may readily gain access to the nerves by blood and lymph channels. Callender and Bitterman conclude that their data on the location of the primary lesion add to similar data reported by others and favor the theory that the organism enters the body through abrasions or similar wounds in the integument of the exposed parts of the body. Rodriguez⁽³⁾ records a single cutaneous lesion, believed to be the initial lesion in 75 per cent of a series of 59 children who became leprous at Culion. The commonest sites of this initial lesion were the buttocks, 28 per cent; extremities, 34.9 per cent;

and cheeks, 11.8 per cent. From this unusually favorable material it seems probable that in 75 per cent of early cases the first demonstrable lesion is not only found upon the skin, but upon an exposed part of the body.

Admitting the probability that the skin is the most frequent portal of entry, we are confronted with a further difficulty. According to the opinion of most authorities leprosy is contagious; that is, it is conveyed by personal contact with a leper, or by intermediate contact by handling articles contaminated by lepers. Some of the known facts concerning leprosy favor this view; as, for instance, the enormous number of bacilli discharged by advanced lepers from ulcerating lesions of the skin, from the nasal lesions, and in the various secretions and excreta; the fact that leprosy is often a familial disease; that the denser the population, the greater is the tendency to the incidence of the disease in those countries where it is endemic; and finally that from time immemorial popular opinion has always assigned contact as the method of transmission.

While not denying that such contact may be one method of transmission of leprosy, there is nothing in the evidence available to indicate that it is the only method or even the most usual method of transfer. In fact there are some considerations that indicate that transfer by bodily contact must be unusual. Hirsch(4) has compiled a great mass of evidence in opposition to the transmission of leprosy by contact, and says:

I am convinced that there is not a single fact that positively and irrefutably shows the transmission of the disease through contagion; on the other hand there are very cogent facts that contradict such a conception since they are in complete contradiction with all experience concerning the transmission of true contagious diseases.

Such arguments may be summarized as follows:

1. The frequently observed instances in which endemic foci are sharply circumscribed in spite of free and even unhygienic communication with the neighboring people. In the Philippines, for example, we have the relatively high infection rate for Cebu and for Albay and the Bicol provinces in spite of free communication with the surrounding people.

2. Although leprosy is often a familial disease, on the other hand there are many more instances where it has not spread to other members of the family. Even married couples, one of whom was leprous, have lived in intimate relationship as long as ten years without conveying the infection, and between in

fected husbands and wives usually not more than 5 per cent contract the disease, the single exception being in India, where the percentage is 6.5.

3. McCoy in Hawaii, Gregory in Cape Colony, and the Leprosy Commission in India found that the proportion of healthy persons living with lepers who became infected is 4.2, 4.5, and 5.5 per cent, respectively, in these different countries, while in Japan and Norway the percentage in both was about 2.7. Thus leprosy cannot be considered as a very contagious disease since only about one person out of twenty, living in close contact with a leper, contracts it. This very small percentage of infected, among people taking no precautions, and continuously exposed, indicates that leprosy is far less transmissible than syphilis, tuberculosis, or any other disease that is known to be transmitted by direct or indirect contact.

4. Doctors and nurses in leprosaria, where they come into daily, intimate, personal contact with lepers and with objects contaminated by lepers, never contract the infection. The few doubtful exceptions that are recorded only prove the rule. Leprosy has never been transmitted even in the numerous instances in which physicians have wounded themselves while operating upon lepers and attending women in labor.(4, 5)

5. All but one of the numerous attempts that have been made to inoculate man experimentally have been completely unsuccessful. Thus, in the years 1844-1858, Danielssen(1) repeatedly inoculated himself, several assistants, doctors, and syphilitic and favus patients, a total number of twenty, with leper nodules, which he placed under the skin, with blood, and with pleuritic exudate, without any positive result. Profeta also inoculated ten persons without result, and similar experiments of Bargilis were negative. One such experiment by Arning(6) was positive, though even this is not fully accepted by all authorities, owing to the fact that the experiment was performed in an endemic area and on a man who, as has been subsequently claimed, came from a leprous family.

As the result of these undoubted facts it must be admitted that if leprosy is transmitted by contact it is only with great difficulty, under very special circumstances, and usually only after long and intimate association with a leper. Children are believed to be especially susceptible to leprosy, yet Rodriguez has found that the average age at which children born of leprous parents and living in a leper colony were found to be positive

was 5 years 9 months. Calculating the incubation period (concerning which nothing can really be known) as three years nine months, the average age of infection would be 2 years. Close and intimate association with a leper for at least two years is therefore required on the average for the transmission of leprosy even to children. One would, therefore, naturally suppose that if leprosy were commonly transmitted by contact, practically all lepers would give a history of previous association with a leper. But this is not the case. McCoy,⁽⁷⁾ who is himself an advocate of personal contact as the cause of leprosy, studied the histories of 1,060 lepers segregated at Molokai. Of these, association with lepers prior to segregation could be demonstrated in only 461, or 43+ per cent; while it could not be demonstrated in 599, or 56+ per cent. Callender and Bitterman, who investigated the histories of 259 lepers at San Lazaro Hospital, Manila, found only 36.9 per cent who admitted any previous contact with lepers, familial or otherwise; Muir⁽⁸⁾ found that of 3,380 Indian lepers, only 58 per cent remembered contact with lepers prior to developing the disease; and similar figures have been obtained by other observers. Making all necessary allowances for ignorance in some cases and the tendency to deny previous association, it would seem that previous contact with lepers should be demonstrable in a higher percentage of cases if the disease were acquired by contact, especially as we have shown that such contact must generally be prolonged and intimate.

Still more convincing are the occasional cases that occur among Americans or Europeans in the Philippines, none of whom has been in intimate association with lepers, and few of whom have any recollection of contact, however casual, with a leper. I have personally known several such cases, and there is the history of one case in a lady, the wife of an officer.

All of these considerations throw doubt upon contact as the usual method of transmission and suggest the possibility that leprosy may more commonly be transmitted by some intermediate agency, such as the bites of certain insects. This possibility has, indeed, been considered by all authors, usually only to be dismissed rather summarily. Thus, McCoy⁽⁶⁾ writes:

The second theory and a most alluring one is that of insect transmission. The fly, the louse, the bed-bug, the mosquito, and the itch-mite have all been under suspicion at one time or another, but when the affirmative evidence in each case has been submitted to careful laboratory and epi-

demiological study it has been found to be inadequate to account for the general transmission of the disease. So far as flies are concerned it cannot be denied that it is conceivable that they might carry leprosy bacilli on their legs, and it has been experimentally shown that they can carry the organisms of rat leprosy and other acid-fast organisms from place to place, but that this can be considered as more than a possible, or at most an occasional, mode of transmission remains to be shown.

With regard to blood-sucking insects the evidence is also flimsy. In the many insects of this sort that have been examined when caught in the environment of lepers or after feeding on leprosy lesions the bacilli have been found so rarely as to make it unlikely that this means of conveyance occurs commonly. The lesions of leprosy are of such a nature that an insect can pierce through them into the blood stream and suck blood, without organisms being imbibed or even contaminating the piercing organs. There is one point in this connection that has not been sufficiently considered; during the febrile periods through which so many lepers pass, the bacilli may circulate in the peripheral blood, at times in large numbers, and it is possible that at such periods blood-sucking insects may take up the bacilli.

With regard to the itch-mite, we can only say that while lepers are very often infested with this parasite, many persons develop leprosy who have never had itch.

These objections are far from convincing. In answer to the statement that, "the blood-sucking insects become infected so rarely as to make it unlikely that this means of conveyance occurs commonly," it may be replied that leprosy is not a common disease, only about one per thousand of the inhabitants of the Philippines becoming infected. If a blood-sucking insect may transmit leprosy, it can only do so by biting a leper through a leproma and so becoming infected. Since the lepra bacilli cannot be supposed to undergo any cycle of development in an insect host, the insect can only transmit the disease mechanically, and must therefore bite another victim while its proboscis is still soiled with viable bacilli. This might readily happen with lice or fleas, which feed several times within twenty-four hours, or with mosquitoes and biting flies that have been interrupted while feeding. The difficulty of transmission in this manner favors this theory of transmission rather than rendering it improbable; for if insects could transmit leprosy as lice transmit typhus, fleas transmit plague, or mosquitoes transmit malaria, enormous numbers would develop leprosy. Again, the statements that "the lesions of leprosy are of such a nature that an insect can pierce them without becoming infected," is undoubtedly often correct. Nevertheless, the larger tubercles, which contain innumerable bacilli, are not so readily

pierced, and personal experiment has shown that lepra bacilli can be demonstrated in 40 per cent of the mosquitoes that are allowed to bite such lesions. If it is only rarely that an insect succeeds in biting over such a favorable area under natural conditions, this again agrees with the epidemiological facts.

On the other hand, a number of considerations may be advanced in favor of the theory of insect transmission. This theory not only accounts for the rarity of the disease, but it explains why long and close association with a leper is usually essential to transmission. It not only explains the increase in incidence of infection with increase in density of population as well as the personal contact theory, but it also explains the exceptions to this tendency, and the prevalence of the initial lesion on an uncovered portion of the body. Noc(1) made a point of this fact together with the appearance of leprosy, in his experience, only in the zones where there were mosquitoes, and demonstrated lepra bacilli in half of one hundred fifty culicine mosquitoes that had bitten advanced lepers. It explains the more frequent infection of those inhabiting the same house with a leper. Still more important, it affords an explanation for the occurrence of leprosy among persons who, so far as known, had no previous contact with lepers, certainly none but a most casual contact, which all experience has shown does not transmit the disease. Still further, the introduction of a few bacilli into the skin combined with the irritating saliva of the insect may afford just the circumstances needful to a successful inoculation, which have been so conspicuously absent in the numerous experimental inoculations on man. Finally, lepra bacilli have been found on numerous occasions and with a considerable degree of frequency in a number of biting insects, as will be seen from the following résumé of the literature, which moreover makes no claim to being complete and exhaustive.

Flies.—Joly(1) in Madagascar found bacilli in the intestinal tract and on the feet of flies that fed upon leprosy ulcers, and believed that they might infect food. Currie(9) examined flies of several species found in the Hawaiian Islands, chiefly *Musca domestica*, and found that when permitted to feed upon leprosy fluids these flies contain lepra bacilli in their intestinal tracts and feces for several days, and that they may convey immense numbers of lepra bacilli to the skin, mucous membranes, and digestive tracts of healthy persons, and that we are justified in regarding these insects with grave suspicion as being one of

the means of disseminating leprous infection. Sandes(10) inclosed flies over ulcerated leprotic surfaces, and out of seventy, found lepra bacilli in the gastro-intestinal tract in two. In 1913 Leboeuf(11) found lepra bacilli in nineteen out of thirty-six flies captured in the rooms of lepers; and in 1916 Marchoux found the bacilli in the digestive tract and on the feet of flies, but that they died rapidly outside of the intestine. In 1917 de Buen,(11) out of a lot of fifty flies fed on ulcerating lepromata, found five slightly infected and twenty-six grossly infected. Mello and Cabral(11) in 1926 found that 40 per cent of the flies (*Musca bezzii*) captured from the leper asylum or fed on lepromata contained lepra bacilli in their intestinal contents, while it was impossible to find any acid-fast bacilli in those flies captured in other places.

Thus there is a very considerable accumulation of evidence that flies become infected with lepra bacilli. However, non-biting flies could only transmit the disease by contact or by infecting food, methods of transmission that we have already considered as incapable of explaining all the known facts concerning the epidemiology of leprosy. In this discussion we are more interested in biting insects, among which bedbugs, lice, fleas, and mosquitoes may be considered.

Bedbugs.—Bedbugs have been incriminated by several writers who have found lepra bacilli in them, while several other investigators have failed to find them.

Goodhue(12) was apparently the first to find lepra bacilli in the common bedbug (*Cimex lectularia*) and writes—

I believe that the *Cimex* is more of a factor in the spread of leprosy among the natives than the gnat, for the following reasons, viz., the bedbug's invasion is noiseless and insidious, made during the sleep of the victim, and secondly, the beds and bedding used in a leper family, whether the leper is dead or segregated, are occupied by clean persons without adequate disinfection.

Ehlers, Bourret, and With (13) examined seventy-one bedbugs that had bitten lepers, in none of which were lepra bacilli found with certainty, although they reported three as doubtful.

Sandes(10) examined seventy-five bedbugs that had bitten lepers and found lepra bacilli in twenty of them, often in considerable numbers (twelve in one field). These bacilli do not readily disappear but can be found up to sixteen days after the insects are fed. They were also found in smears made

from the mascerated head and proboscis five days after feeding. Acid-fast bacilli were not found in bugs collected from non-leper sources. Sandes concludes that there is reason to believe that the bedbug constitutes a very important agent in the transmission of leprosy.

Long(14) also regularly found lepra bacilli in bugs that had bitten lepers, while control bugs were negative. Long also reported a case in a native who so far as known had never been in direct contact with a leper, but who had on several occasions slept in the bed of a leper in a different town and was severely bitten by bugs there.

Skelton and Parham(15) examined seventy-five bedbugs caught on beds at a leper asylum and with one doubtful exception found no lepra bacilli in them. This, however, is quite a different experiment from permitting the bugs to bite lepers. However, Thompson(16) fed bedbugs on cases of leprosy on several occasions, and of one hundred one bugs so fed, was unable to find lepra bacilli in any.

Again, however, lepra bacilli were definitely found by Johnston(17) five times out of three hundred fifteen bugs collected at Culsion from the beds and houses of lepers. Only fifteen bugs were examined by direct smear, and acid-fast bacilli were found in one.

According to Mello and Cabral, de Buen in 1917 found bedbugs to be infected frequently, the percentage being 9.9 among bedbugs fed upon nodular lepers, and 0.88 among bedbugs collected in the beds of leper patients.

Mello and Cabral(11) themselves also investigated bedbugs and found that out of twenty bedbugs collected from the beds of lepers at the asylum of Mapuca, ten contained lepra bacilli in the intestinal tract. Thirty-six bedbugs were permitted to bite cases of nodular leprosy whose nodules had previously been shown to contain bacilli. Lepra bacilli were found in twenty-one, the number varying from one to twelve, and in the latter cases five globi were also found. Bedbugs from nonleprosy sources were always negative. They also found that after an infective feed, the number of lepra bacilli commenced to diminish toward the fourth day.

Rodriguez(3) suggests the possibility of the bedbug as a transmitting agent, stating that acid-fast organisms considered to be lepra bacilli, have been found in 10 per cent of three hun-

dred two bedbugs fed on lepers, as reported by various workers, and 1 per cent of five hundred sixty-six specimens caught from lepers' quarters.

In the case of the bedbug, the evidence is conflicting, but there have been so many different positive findings that they must be considered as outweighing the negative findings. Not only do positive findings as a rule carry more weight than negative findings, but this is especially the case in an investigation of this character. A slide may be searched for an hour for lepra bacilli without result, and finally a definite clump may be found when the searcher is about to discontinue the search and call it negative. Obviously the more carefully nodules are selected, so that the bugs bite over an area containing numerous bacilli and the more painstaking the subsequent examination of the bugs, the higher will be the percentage in which lepra bacilli will be found in the intestinal contents.

Lice.—Joly(18) cites Sabrazés as suspecting lice of transmitting leprosy, and noted the prevalence of lice among the poorer classes in Algeria, these classes furnishing the greater number of lepers in that country.

Ehlers, Bourret, and With(13) examined twenty lice that had bitten lepers, of which nineteen were negative, while one was classed as doubtful. They found "three or four acid-fast, extra cellular bacilli," which they did not regard as characteristic lepra bacilli. However, it seems probable that they were, since no such acid-fasts were found in lice that did not bite lepers.

McCoy and Clegg(19) state:

In some recent work in connection with the study of the possibility of the transmission of leprosy by animal parasites, we found a large number of acid-fast bacilli in smears made from two lice (*Pediculus capitis*) taken from an advanced case of nodular leprosy. In morphology, grouping, and tinctorial characteristics, the organisms found in these insects were indistinguishable from the leprosy bacillus. There was a leproma on the forehead adjacent to and invading the scalp . . . We have examined lice from several other cases of leprosy but with uniformly negative results.

From the above, it will be seen that although the evidence that lice may contain leprosy bacilli is not so extensive as for some other insects, the statement by McCoy and Clegg is very definite. We must assume that under the proper circumstances lice do become infected, and the louse must be considered a possible though probably not frequent vector of leprosy.

Fleas.—Fleas are very difficult to handle and to keep alive, and relatively few examinations of them have been made,

yet these are quite suggestive. Sandes⁽¹⁰⁾ examined sixty fleas that had bitten lepers and found acid-fast bacilli corresponding to lepra bacilli in two of them. Ehlers, Bourret, and With⁽¹³⁾ found lepra bacilli in one out of thirty-one fleas fed upon lepers.

Mosquitoes.—Leloir (Traite pratique et theoretique de la lepre, Paris, 1886) believed mosquitoes to be possible vectors of the infection. Arning (Archiv. f. Dermat. und Syph. 1891, No. 1, Congress de Berlin) remarks that leprosy and mosquitoes invaded the Hawaiian Islands at almost the same time. Blanchard (Bull. de l'Acad. de Med. July 30, 1905) states that leper countries are also usually mosquito countries. Hallopeau (Bull. de l'Acad. de Med. July, 1901) and Chantemesse (Bull. de l'Acad. de Med. July 30, 1901) favored the hypothesis that leprosy is transmitted by culicines.⁽²⁰⁾ These writers based their argument upon theoretical considerations.

Noc (Ann. d'Hyg. et de Med. Col. July–Sept. 1903, p. 481) not only pointed out that the first lesion of leprosy usually occurs on uncovered portions of the body, and that lepers usually only occur in countries where mosquitoes are numerous, but examined mosquitoes and found lepra bacilli in half of one hundred fifty culicine mosquitoes.

Goodhue⁽¹²⁾ found lepra bacilli in considerable numbers in a few out of a large number of *Culex pungens* caught at random from leper houses. Ehlers, Bourret, and With⁽¹³⁾ examined twelve *Stegomyia fasciata* Fabricius (*Aedes aegypti* Linnæus) and found in one not only numerous single lepra bacilli, but also a globus. Currie⁽⁹⁾ examined four hundred ninety-three mosquitoes collected from the rooms of lepers and found no acid-fast bacilli in any of them. He concludes, therefore, that mosquitoes feeding under natural conditions, imbibe lepra bacilli so rarely that this insect is of no importance in the transfer of the disease. This conclusion cannot be accepted, for Currie's negative results are balanced by the positive results of Goodhue which were also obtained on mosquitoes collected at random. As Currie himself says, if mosquitoes do contain the bacilli, the mechanism for the transfer of the bacilli is complete and the insect *may* be a factor in the transmission of the disease.

Supposing mosquitoes to be the usual method of transfer, the fact that they become infected so rarely under natural conditions of biting, is a good explanation of the fact that only 5 per cent of the persons in close association with lepers ever develop leprosy.

Ticks.—According to Mello and Cabral⁽¹¹⁾ ticks have been examined in Brazil by Rodolpho who has found lepra bacilli in their intestines as long as thirteen days after a feed upon a patient suffering from leprotic fever.

Sarcoptes scabiei has been suggested as a probable cause by several investigators, but without finding lepra bacilli on them. Most writers dismiss this possibility with the statement that most lepers have never had the itch. This by no means excludes the probability that infection may at times be carried by this parasite, whose known habits strongly favor this possibility. Such a method of transfer would explain the case quoted by Manson-Bahr⁽²¹⁾ "as an instance that can only be explained by contagion." This case was an Irishman who had acquired the disease in the West Indies. After his return to Ireland, he slept in the same bed as his brother, who moreover, sometimes wore the leper's clothes. This brother, who had never been out of the United Kingdom, became a leper.

Rodriguez⁽³⁾ reports three cases in which the first lesion of leprosy developed from a scabies scar. This is fairly convincing evidence that infection may occur in this manner.

Demodex folliculorum.—Borrel⁽²²⁾ suggested this parasite as a transmitting agent. Ehlers, Bourret, and With⁽¹³⁾ found in their sections of lepromata numerous hair follicles containing free lepra bacilli and epithelial cells containing globi. At one place they found a *Demodex* surrounded by a great quantity of lepra bacilli, and believed that their findings afforded support for Borrel's hypothesis that the *Demodex* might convey the infection.

From this summary of the literature it is evident that leprosy *may* be transmitted by a wide variety of biting insects and skin parasites. It may well be that no one of these parasites is the sole transmitting agent, but that several or all of them may serve to transmit the infection under favorable circumstances. The familiar tendency of the disease, the value of isolation, the rarity of the infection among those exposed, the location of the first lesion on an exposed part of the body, and even cases cited as sure proof of the contagion theory can be readily explained by assuming transmission by one or more of these insects. Considering also the great difficulty in explaining the incidence of the disease on the theory of contagion, it would seem that

more authorities should agree with Castellani and Chalmers⁽²³⁾ who state that "everything in the history of the disease appears to us to favor its spread by animal agency."

EPIDEMIOLOGICAL OBSERVATIONS

Desiring to obtain any information that might throw light upon the method of transmission of leprosy, a study was made of the incidence of leprosy in the Philippines. Since the actual number of lepers is not known with certainty, the study was based upon 16,551 lepers diagnosed and segregated during the twenty-one years 1906-1926. A tabulation of these lepers, by provinces, was furnished me through the courtesy of Dr. Lopez Rizal, of the Philippine Health Service, together with the estimated population of all the provinces during these years. From these figures, the average annual admission rate per thousand was worked out for each province (Table 1).

GEOGRAPHICAL DISTRIBUTION

The incidence of leprosy by provinces is very irregular. Albay, Sorsogon, and Camarines Sur have high rates (0.156, 0.135, 0.080), while Camarines Norte and Tayabas, adjoining, have low rates (0.030, 0.035); Zambales, Bulacan, and Nueva Ecija have relatively high rates (0.169, 0.090, 0.067), while the intervening provinces Tarlac, Pampanga, and Pangasinan have the low rates of 0.041, 0.039, and 0.016; Ilocos Sur has a rate of 0.022, while Ilocos Norte on one side has a rate of 0.045 and La Union on the other side a rate of 0.057. These examples show that leprosy does not spread evenly among the population by contiguity, but that there are local conditions operative which produce a high incidence of leprosy in certain provinces while people in neighboring provinces who are separated only by an imaginary line have a low incidence.

It has been shown moreover that this distribution is not temporary but has always existed. The disease was especially prevalent in the same localities before the Spanish colonization, and under the Spanish administration lazarettos were early established in Cebu and in Palestina, near Naga, Camarines Sur, as well as in Manila. If leprosy were transmitted solely by contact, it is difficult to understand this irregular distribution among people of neighboring provinces who intermingle freely with each other.

HUMIDITY

It was suggested by Rogers⁽²⁴⁾ that the incidence of leprosy in India depended upon rainfall and humidity and was relatively rare in dry climates. No part of the Philippines is dry, and Guerrero⁽²⁵⁾ showed clearly that this factor was unimportant in the Philippines. Baguio, Mountain Province; Surigao, Mindanao; and Paracale, Camarines Norte, are the stations having the highest average relative humidity, yet they have very low rates of leprosy incidence (0.015, 0.009, 0.030). On the other hand, the stations at Cebu, Vigan, Manila, and San Isidro, which record the lowest average humidities, are in provinces or regions where the rates are among the highest. Twenty-seven per cent of all the lepers in the Philippines come from the small island Cebu.

DENSITY OF POPULATION

From the Statistical Bulletin, published by the Department of Commerce and Communications, the areas of the provinces in square miles were obtained, and the average population per square mile for each province was calculated for the same years (1906-1926). The figures so secured, together with the average annual leper rate per thousand, are shown in Table 1. The average leper rate per thousand was then charted by provinces in the order of density of population as shown in fig. 1. From this analysis two deductions may be drawn, namely:

1. As has been previously stated by several authorities and especially by Guerrero,⁽²⁵⁾ there is a definite tendency for leprosy to increase with density of population. Thus provinces having from 9 to 68 persons per square mile had an average annual admission rate of 0.042 per thousand, in provinces having 101 to 199 persons per square mile the similar rate was 0.068, in provinces having from 222 to 278 persons per square mile the rate was 0.083, while with a population of 305 and upward the rate was 0.140.

2. A glance at the chart (fig. 1) will show that the curve is exceedingly irregular, some localities having a dense population (La Union 448, Pampanga 305, Pangasinan 278, Batangas 255, Marinduque 156) had exceedingly few lepers admitted annually, and Manila, undoubtedly the most densely populated area in the Philippine Islands, had a lower rate than Cebu, Ilo-

FIG. 2. Average annual leper admission rate per thousand. Provinces arranged in order of percentage of cultivated land.

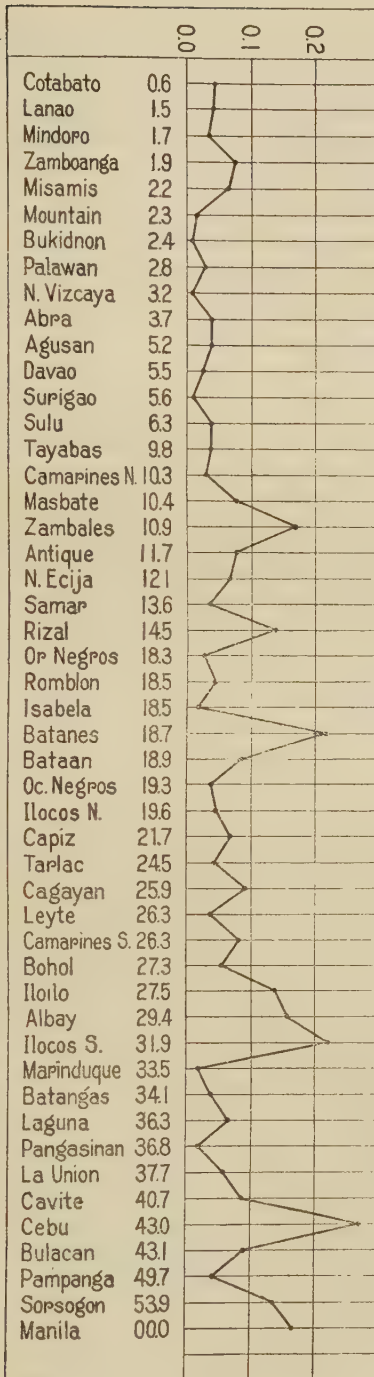
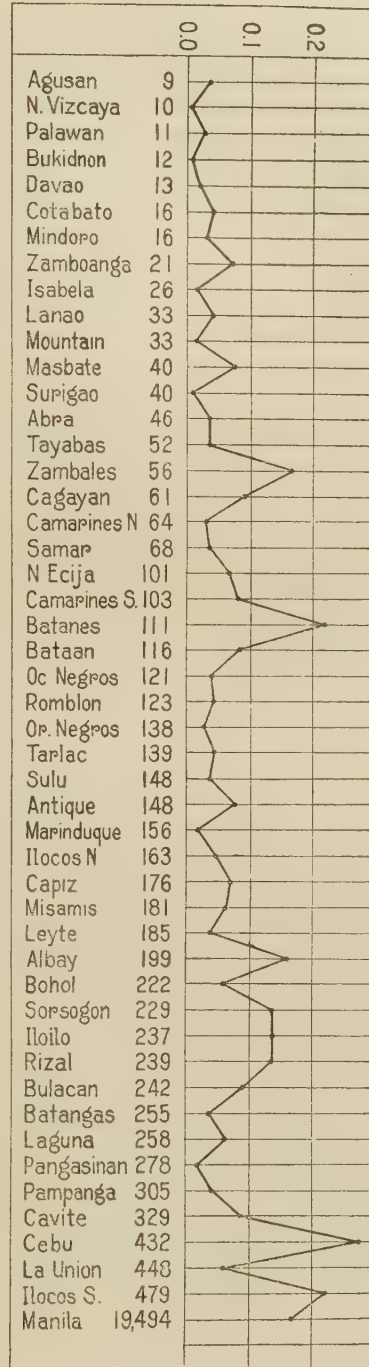


FIG. 1. Average annual leper admission rate per thousand for each province. Provinces arranged in order of density of population.



cos Sur, of Batanes. Cebu, which is very densely populated (432 per square mile), had the highest rate of all, a rate higher than the rate for Manila whose population is 19,494 per square mile. On the other hand, a number of provinces having a comparatively sparse population (Zambales 56, Batanes 111, Albay 162) had unusually high admission rates.

This indicates that density of population is only one factor and not the most important factor in the transmission of leprosy, a fact that is inconsistent with the contact theory; for if leprosy were transmitted always or even usually by contact, the rate of infection should increase more regularly with density of population.

Population is not evenly distributed with the same number of inhabitants for each square mile as assumed in fig. 1, and several attempts were made to determine the population distribution more accurately.

Thus, it may be assumed that more people live upon cultivated than upon uncultivated land. The percentage of cultivated land in each province as given by the Statistical Bulletin was arranged in order from the lowest to the highest and charted with the leper rate for the corresponding provinces (fig. 2). The curve of leper incidence plotted in this way agrees in general with fig. 1 and shows the same irregularities.

Figure 3 was then prepared on the assumption that in provinces having less than 7 per cent of cultivated land the population would be evenly distributed, and that in provinces having more than 7 per cent the entire population would be concentrated on the cultivated land.

Land is classified by the Statistical Bulletin as commercial forest, noncommercial forest, swamp land, cultivated land, and grassland. Inhabitants cannot be numerous in forest except in the case of the uncivilized tribes. Therefore, fig. 4 was prepared on the assumption that in provinces having less than 20 per cent cultivated land and grassland the majority of the population was evenly distributed, and that in provinces having more than 20 per cent cultivated land and grassland the total population lived upon this land. Figures 3 and 4 agree in general with figs. 1 and 2 and are quite as irregular. In fact, the closer we approach the actual density of the population, the more irregular becomes the curve of the leprosy incidence.

FIG. 4. Leper rate charted on assumption that population lives on cultivated land and grassland.

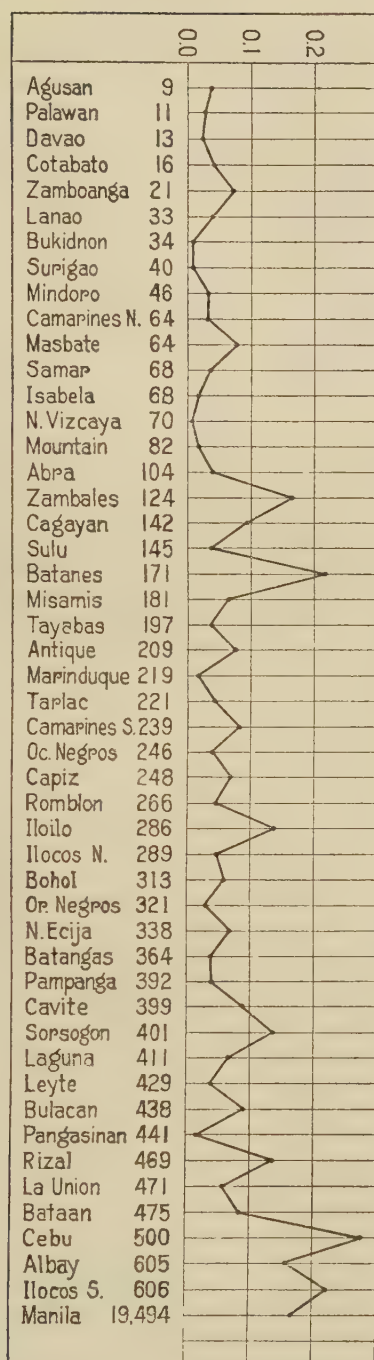


FIG. 3. Leper rate charted on assumption that population lives on cultivated land.

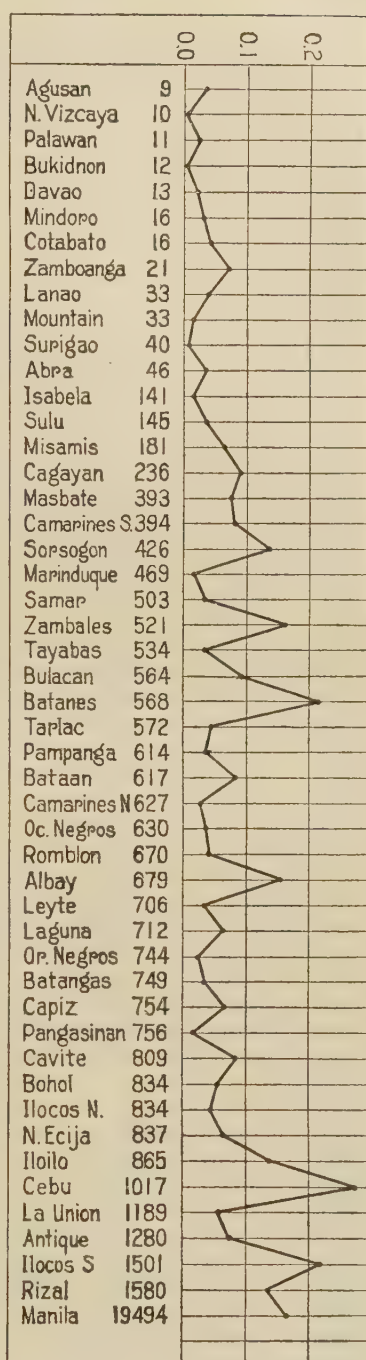


TABLE 1.—*Provinces of the Philippines arranged alphabetically and showing for each the total population, the area in square miles, the population per square mile, and the average annual leprosy admission rate.*

Province.	Average population, 1906-1925.	Area in square miles.	Population per square mile.	Annual leper rate per thousand.
Abra.....	68,776	1,475	46	0.037
Agusan.....	40,980	4,294	9	.035
Albay.....	307,589	1,543	199	0.156
Catanduanes.....				
Antique.....	151,100	1,011	148	0.074
Bataan.....	56,150	480	116	.083
Batanes.....	8,214	74	111	0.218
Batangas.....	324,572	1,270	255	0.037
Bohol.....	341,492	1,536	222	0.058
Bukidnon.....	48,544	3,871	12	0.008
Bulacan.....	244,450	1,007	242	0.090
Cagayan.....	183,707	3,007	61	0.090
Camarines Norte.....	50,176	779	64	0.030
Camarines Sur.....	214,900	2,072	103	0.080
Capiz.....	279,928	1,710	176	0.068
Cavite.....	153,077	464	329	0.088
Cebu.....	816,917	1,867	432	0.275
Cotabato.....	163,242	9,620	16	0.041
Davao.....	100,126	7,486	13	0.021
Ilocos Norte.....	211,524	1,293	163	0.045
Ilocos Sur.....	211,747	442	479	0.220
Iloilo.....	485,397	2,040	237	0.137
Isabela.....	106,038	4,052	26	0.016
Laguna.....	186,651	722	258	0.062
Lanao.....	81,982	2,439	33	0.040
La Union.....	156,941	350	448	0.057
Leyte.....	558,344	3,005	185	0.035
Manila.....	272,922	14	19,494	0.165
Marinduque.....	55,884	356	156	0.017
Masbate.....	62,995	1,545	40	0.079
Mindoro.....	65,801	3,928	16	0.031
Misamis.....	186,683	1,030	181	0.061
Mountain Province.....	215,345	6,447	33	0.015
Nueva Ecija.....	209,484	2,069	101	0.067
Nueva Vizcaya.....	35,838	3,530	10	0.005
Occidental Negros.....	379,893	3,125	121	0.039
Oriental Negros.....	259,065	1,902	136	0.027
Palawan.....	62,732	5,619	11	0.029
Pampanga.....	251,201	823	305	0.039
Pangasinan.....	540,888	1,944	278	0.016
Rizal.....	215,502	899	239	0.135
Romblon.....	62,382	505	123	0.041
Samar.....	358,152	5,234	68	0.034
Sorsogon.....	167,465	729	229	0.135
Sulu.....	157,203	1,082	145	0.034
Surigao.....	116,110	2,889	40	0.009
Tarlac.....	164,911	1,178	139	0.041
Tayabas.....	200,847	3,839	52	0.035
Zambales.....	80,888	1,421	56	0.169
Zamboanga.....	138,000	6,383	21	0.072

TABLE 2.—*Provinces of the Philippines arranged by per capita income from nine principal crops and fishing and showing the annual average leprosy admission rate for each province.*

Province.	Per capita income.	Annual admission rate for leprosy per thousand.	Province.	Per capita income.	Annual admission rate for leprosy per thousand.
	<i>Pesos.</i>			<i>Pesos.</i>	
Batanes.....	6	0.218	Oriental Negros.....	37	0.027
Sulu.....	6	0.034	Ilocos Norte.....	39	0.045
Cotabato.....	9	0.041	Misamis.....	42	0.061
Palawan.....	10	0.029	Mindoro.....	45	0.031
Rizal.....	15	0.135	Cagayan.....	46	0.090
Masbate.....	17	0.079	Pangasinan.....	48	0.016
Antique.....	19	0.074	Surigao.....	49	0.009
Bukidnon.....	19	0.008	Albay.....	50	0.156
Bohol.....	22	0.058	Tarlac.....	51	0.041
Zambales.....	22	0.169	La Union.....	52	0.057
Mountain Province.....	23	0.015	Sorsogon.....	53	0.135
Samar.....	24	0.034	Camarines Sur.....	54	0.080
Cebu.....	25	0.275	Isabela.....	54	0.016
Zamboanga.....	27	0.072	Agusan.....	58	0.035
Batangas.....	27	0.037	Marinduque.....	59	0.017
Lanao.....	28	0.040	Nueva Vizcaya.....	65	0.005
Romblon.....	31	0.041	Bataan.....	66	0.083
Leyte.....	32	0.035	Camarines Norte.....	70	0.030
Abra.....	33	0.037	Pampanga.....	82	0.039
Bulacan.....	34	0.090	Tayabas.....	84	0.035
Ilocos Sur.....	36	0.220	Davao.....	87	0.021
Capiz.....	37	0.068	Laguna.....	95	0.062
Cavite.....	37	0.088	Nueva Ecija.....	95	0.067
Iloilo.....	37	0.137	Occidental Negros.....	162	0.039

These illustrations, which might be multiplied, show that some local condition other than contiguity or density of population is the most important factor influencing the transmission or nontransmission of leprosy. Lepers exist in all of the provinces, but in some, especially Cebu, Ilocos Sur, Albay, Batanes, and Zambales, conditions other than density of population facilitate the transmission of the disease.

WEALTH

The Statistical Bulletin gives the total annual value of the nine principal crops (rice, abacá, sugar cane, tobacco, coconuts, corn, maguey, cacao, coffee), together with the annual income from the fishing industry, by provinces. From these figures the per capita annual income was calculated for each province (Table 2). The provinces were then arranged in order from the lowest to the highest and charted with their average annual leper rates (fig. 5). From this it will be seen that there is a

FIG. 6. Leper rates charted by provinces. Provinces arranged in order of the ratio between density of population and per capita income.

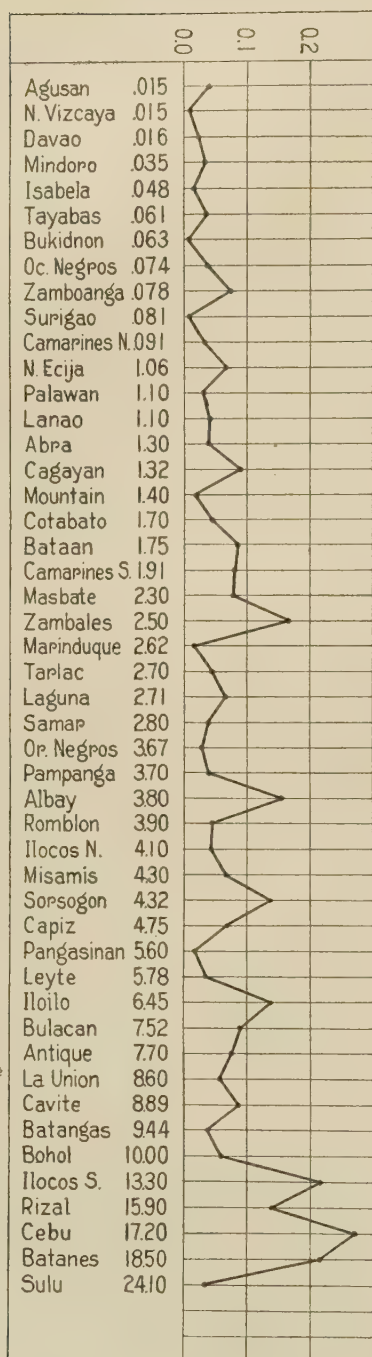
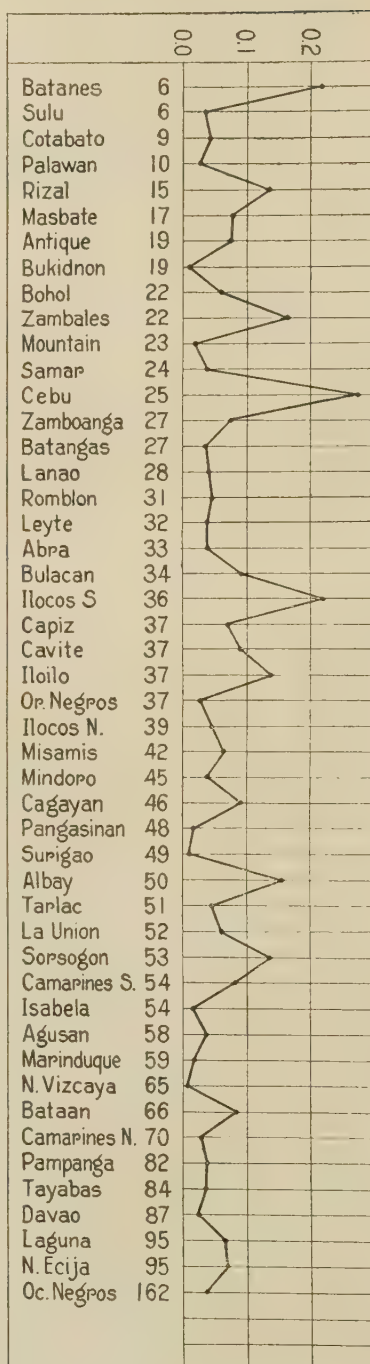


FIG. 5. Leper rates charted by provinces arranged in order of per capita annual income



distinct tendency for the occurrence of higher leper rates among the poorer people. If the forty-eight provinces are equally divided, the first twenty-four, receiving the lower incomes, have an average annual leprosy rate of 0.083; while the twenty-four provinces receiving the higher incomes have an average annual rate of 0.049.

This might be reasonably expected since, other things being equal, well-to-do people live under conditions of better sanitation and have a better diet than poor people, but the chart again is very irregular and shows clearly that this is a minor factor governing the incidence of the disease. These irregularities are not in the least corrected even when both factors of density of population and annual income are taken into consideration, as shown by fig. 6 in which the provinces are charted in the order indicated by a ratio between density of population and per capita income.

TABLE 3.—*Provinces of the Philippines arranged alphabetically and showing for each the beriberi mortality rate, 1920–1924, and the average annual leprosy admission rate.*

Province.	Mortality rate from beriberi per thousand, 1920–1924.	Admission rate for leprosy per thousand.	Province.	Mortality rate from beriberi per thousand, 1920–1924.	Admission rate for leprosy per thousand.
Abra.....	0.168	0.037	La Union.....	0.996	0.057
Agusan.....	0.509	0.035	Leyte.....	1.698	0.035
Albay.....	2.315	0.156	Marinduque.....	2.447	0.017
Catanduanes.....			Masbate.....	0.439	0.079
Antique.....	0.670	0.074	Mindoro.....	2.883	0.031
Bataan.....	3.547	0.083	Misamis.....	2.293	0.061
Batanes.....	0.243	0.218	Mountain Province.....	0.131	0.015
Batangas.....	2.620	0.037	Nueva Ecija.....	4.344	0.067
Bohol.....	0.759	0.058	Nueva Vizcaya.....	0.463	0.005
Bukidnon.....	0.308	0.008	Occidental Negros.....	1.076	0.039
Bulacan.....	4.299	0.090	Oriental Negros.....	1.665	0.027
Cagayan.....	1.098	0.090	Palawan.....	0.130	0.029
Camarines Norte.....	1.557	0.030	Pampanga.....	2.945	0.039
Camarines Sur.....	1.559	0.080	Pangasinan.....	1.077	0.016
Capiz.....	0.891	0.068	Rizal.....	3.398	0.135
Cavite.....	5.131	0.088	Romblon.....	0.788	0.041
Cebu.....	1.202	0.275	Samar.....	1.196	0.034
Cotabato.....	0.071	0.041	Sorsogon.....	1.113	0.135
Davao.....	0.883	0.021	Sulu.....	0.057	0.034
Ilocos Norte.....	0.736	0.045	Surigao.....	0.797	0.009
Ilocos Sur.....	0.615	0.220	Tarlac.....	2.366	0.041
Iloilo.....	0.578	0.137	Tayabas.....	2.173	0.035
Isabela.....	0.859	0.016	Zambales.....	2.009	0.169
Laguna.....	3.073	0.062	Zamboanga.....	0.910	0.072
Lanao.....	0.097	0.040			

Muir(26) has stated that for India the most outstanding determinant of leprosy is dietetic. In all the regions where the incidence of leprosy was high there was a shortage of essential foods leading to partial starvation, which was usually associated with the consumption of decomposing food, especially bad fish. A bad diet must undoubtedly be another predisposing cause of leprosy, but cannot be the main determining factor in the Philippines. Without denying that the diet of many Filipinos is deficient, a fact that is indicated by the incidence of beriberi, there is no such difference in the food habits of the people of the different provinces as would account for a leper admission rate of 0.156 in Albay as compared with 0.037 in Batangas, or 0.08 in Camarines Sur and 0.03 in Camarines Norte; nor does the incidence of beriberi show any real correlation with the leper rates, as indicated by Table 3.

None of the factors that have been considered can be the main factor influencing the prevalence of leprosy, nor can it be expected that we can determine the conditions responsible for the transmission of the disease from such limited data. So far as it goes, the evidence is against transmission by contact and is so far favorable to the hypothesis that leprosy is an insect-borne disease.

EXPERIMENTAL

EXAMINATION OF MOSQUITOES

While it has been shown that several insects may be possible vectors of leprosy, it was believed that mosquitoes should be investigated first; since, if the disease is insect borne, the cases of leprosy that occur without known contact with previous lepers can be explained best by infection through a flying, biting insect. The following method was first employed to determine the percentage of mosquitoes that might be infective after biting lepers.

Aedes aegypti was selected because the insects of this species bite freely during the day, and the mosquitoes were raised in the laboratory from the eggs. Freshly emerged females that had never bitten were collected in test tubes, one mosquito in each tube, and taken to San Lazaro Hospital where they were permitted to bite lepers. The lepers were selected cases of macular and tubercular leprosy. A suitable area on the skin of the leper was selected, and examined microscopically. Only if many bacilli were present in the area selected were the mos-

quitoes permitted to bite. The test tube was then applied over this area, and the mosquito allowed to bite until filled with blood. In some instances the mosquito was interrupted and allowed to bite several times before it had completed its feed. I am indebted to the director of the hospital and to Doctor Tietze and Doctor Pardo for the facilities afforded for this work. Ten different lepers were used on different days, allowing about ten mosquitoes to bite on different favorable areas on each leper. As soon as the mosquitoes had all bitten, they were carried in the stoppered test tubes to the laboratory and killed by dropping chloroform upon the stoppers. The wings and the legs were then removed, and the blood from the abdomen was expressed and spread upon a glass slide. These slides were then stained in the usual way with carbol fuchsin, decolorized with 20 per cent nitric acid and alcohol, counterstained with methylene blue, and examined for *lepra bacilli*.

Undoubted *lepra bacilli* were found in 41 per cent of this series of approximately one hundred mosquitoes. They were recognized as *lepra bacilli* not only because they were acid-fast and of characteristic morphology and size, but because of their characteristic grouping. In several instances entire globi were found. No acid-fast organism of any kind was found in ten control mosquitoes that were similarly examined after being permitted to bite normal men.

During the course of this work it appeared that mosquitoes were more apt to become infected if they bit over tubercles where they were obliged to insert the proboscis through a thick leprous infiltration in order to obtain blood, or when they were interrupted so that they were obliged to bite several times. With the experience so gained, it would perhaps be possible to obtain infection of the mosquito in nearly 100 per cent of cases, by proper selection of lepers and technic of biting. However, in a state of nature comparatively few mosquitoes would become infected, so that the precise percentage infected by this experimental method is an academic question.

The important point is that this experiment confirms the observations of others that have been quoted, and shows that under proper circumstances mosquitoes certainly might transmit the disease.

It is not known how long *lepra bacilli* in the proboscis of the mosquito would remain viable, but from the nature of the organism it may certainly be assumed that they would remain

viable for at least several hours, and that if during this period the mosquito bit another victim, as it would surely do if interrupted in its first feed, these lepra bacilli would almost certainly be injected with the saliva of the mosquito into the second host. This mechanism possesses all the points necessary to explain the transmission of a disease having the known characteristics of leprosy, especially the fact that it is transmitted only with great difficulty.

TRANSMISSION EXPERIMENT

Leprosy has never been successfully transmitted to animals, and experimentally only once to man. Accurate information concerning the method of transmission might be of the greatest value in the control and eventual eradication of the disease. Insect transmission is at least possible and the considerations presented above indicate that it is even probable. For these reasons a human experiment was believed to be justifiable.

This proposition was presented to Governor-General Wood, who after due consideration authorized the experiment. Accordingly, three volunteers were secured from Bilibid. The nature of the experiment was explained to these men who agreed in writing to submit to inoculation, and it was promised that should they develop leprosy they would be released from Bilibid and sent to Culion where they would receive treatment. Since they would be kept under constant observation during the experiment, the disease would be detected early should it occur and the prospect of cure would be excellent. Only long-term prisoners were selected who had little to lose and much to gain, for the Board of Pardons had assured me that they would give these men favorable consideration in view of their coöperation in this experiment. It would seem to most people that not many would volunteer for such an experiment, but on the contrary there was a considerable number of volunteers; many were unsuitable for one or more reasons. The essential conditions to the acceptance of a volunteer were as follows:

1. He must freely volunteer, knowing the nature of the experiment.
2. He must be a long-term prisoner.
3. He must be a young man in perfect health.
4. There must be no leprosy in the family nor any history of contact with lepers. He must have been at least two years in isolation from possible infection in Bilibid, to exclude the

possibility of the development of leprosy from any other source than the experiment.

It was determined to use one man as a control, using an intradermal method of inoculation, a method that so far as known had not been used in previous human experiments. The other two men were used to test the possibility of mosquito transmission. The protocols of the experiments follow:

I. José Malaborbor, Bilibid prisoner, 24 years old, sentenced to life imprisonment for robbery and homicide. He was born in Santo Tomas, Batangas. Father and mother are living and well, and four brothers and two sisters are living and well. No lepers in the family. States that he saw a leper once in 1910, but denies any contact with lepers at any time. Has been in Bilibid for the past four years (since 1923).

Personal history.—Has always been well except that he had a fever of some kind in 1915. No history of other illness.

Physical examination.—Figure slight but muscular and apparently well nourished. Skin shows numerous tattoo marks, and scars on the knees, but no eruption of any kind and the skin is otherwise normal. No thickening of the nerves, no anæsthesia, nose and throat normal. No abnormalities of any kind detected. Photographs made, front and rear views.

Inoculation.—March 29, 1927. The inoculation was made using material from the arm of Basilio Lat, an untreated leper from San Lazaro, but without any contact between leper and prisoner other than the inoculation. A slide made from a tubercle on the arm of Basilio Lat showed large numbers of lepra bacilli and globi. This tubercle was incised and scraped with a scalpel, and the scrapings were placed in a sterile watch glass where they were diluted with 0.1 cubic centimeter of sterile salt solution. A small portion of this bloody fluid was spread on a slide and stained, showing numerous scattered lepra bacilli. This material was then taken up in a sterile syringe and with a fine needle injected into the skin of the back of Malaborbor, in the mid line one-half inch above a tattooed mark. A large white plaque formed immediately about the needle, showing that the injection was truly intracutaneous. Operation completed at 9.50 a. m.

March 30–31, 1927. Negative. No acute infection.

April 16, 1927. Three pinpoint papules over area of inoculation.

May 19, 1927. Papules gone. Skin normal.

Monthly examinations made thereafter to include June, 1928, have been completely negative. No local or general symptoms of leprosy.

II. Alfonso Par, Bilibid prisoner, 26 years old with a 27-year sentence. He was born in Unisan, Tayabas. Father living and well. Mother died in 1919, cause unknown. No leprosy in the family, and so far as known he has never seen or been in contact with a leper. He has been in Bilibid during the past nine years. He has never had any kind of illness.

Physical examination.—A very robust, muscular man, who is employed as a blacksmith. There are scars on the right thigh, waist, and left side from injuries, a tattoo mark on the right arm, and scars from acne on the face. Skin oily with numerous comedones, but otherwise normal. No thickening of nerves, no anæsthesia, nose and throat normal. No other abnormalities detected. Photographs made, front and rear views.

Inoculation.—April 18, 1927. Two untreated lepers from San Lazaro were examined, and suitable tubercles were selected in both cases that contained many lepra bacilli as shown in smears. These lepers were then brought to a laboratory in the Bureau of Science. Alfonso Par was also brought from Bilibid to the same room, but the lepers and the prisoner were seated on chairs at opposite sides of the room and were never in the slightest contact. Freshly emerged mosquitoes (*Aedes aegypti*) were caught in sterile test tubes. A mosquito was then permitted to bite one of the selected areas on one of the lepers, and when it was about half filled, it was dislodged by shaking the tube; the tube was plugged and carried at once to the prisoner. The presumably infected mosquito was then permitted to bite the prisoner on the skin of the back in the mid line between the shoulder blades. This site was chosen because the skin is unusually thick in this locality, and because it is a situation that cannot be easily reached so that no feigned eruption could be produced. The mosquito was permitted to feed upon the experimental subject until it was satisfied and left the skin. At times, the mosquito was interrupted in its feed, and allowed to bite a second or even a third time before completing its meal. This process was repeated with twenty-one mosquitoes, the operation requiring about three hours, when the prisoner was returned to Bilibid. The mosquitoes used

were then dissected and the contained blood smeared on slides to be examined later for the presence of lepra bacilli.

This operation was repeated on April 19, when twenty-one mosquitoes were again permitted to feed alternately upon one of the lepers and the prisoner. On each day half of the mosquitoes were permitted to bite each of the lepers. Therefore, at the conclusion of the experiment, the prisoner had been bitten by forty-two mosquitoes that had just bitten a leper over an area known to be infective, twenty-one mosquitoes from each leper. All of the mosquitoes were applied to the prisoner over a small area, of approximately 2 square inches, in the mid line of the back, an area that could be readily recognized subsequently, should any lesion develop.

This prisoner has been kept under continuous observation, being examined monthly, but up to June 30, 1928, fourteen months later, has developed no sign of leprosy, and has remained entirely well and continuously employed at his trade of blacksmith.

III. Benito Lavandilla, Bilibid prisoner, 21 years old, sentenced to life imprisonment for theft with violence and murder. His father was killed in a fight, his mother is living and well. One brother and one sister also living and well. No leprosy in the family, and no previous contact with lepers known. Has been in Bilibid five years.

Physical examination.—A young man of slight physique, but well nourished and healthy, and employed as a clerk. Skin normal, no thickening of nerves, no anæsthesia, nose and throat normal. No abnormalities detected.

Inoculation.—June 7, 1927, was bitten by twenty-three freshly emerged mosquitoes (*Aedes aegypti*) that had previously bitten Basilio Lat, the leper used in Experiment I, and following precisely the technic described in Experiment II. Lat had been under treatment with chaulmoogra for a month, and the tubercles on the skin were much reduced, and slides showed that many of the bacilli had become granular.

June 9, 1927. Again bitten with thirty mosquitoes fed back and forth with Quisquino, a newly admitted and untreated leper whose lesions showed many bacilli.

June 24, 1927. Again bitten with twenty-nine mosquitoes fed back and forth with Alcantara, a newly admitted and untreated leper of several years standing and having tubercles containing many lepra bacilli.

On July 1 the skin over the area on the back where the mosquitoes had been applied was normal. This prisoner has been kept under continuous observation, and examined monthly, but up to June 30, 1928, has developed no sign of leprosy. He remained in perfect health until April 20, when he developed an acute diarrhoea of unknown origin, but probably some infection. Stool examination was negative except for the finding of several hookworm eggs. Recovered in a few days and has been well since (June 30, 1928).

This experiment cannot yet be called negative, for none of the three prisoners has been under observation long enough to exclude the possible development of leprosy. The notes have been compiled at this time because of my immediate departure to the United States, but the observation will be continued by my successor.

It should be noted, that even if this experiment should remain completely negative, it would in no wise disprove the theory that leprosy may be transmitted by insects. It does not even prove that the disease is not transmitted by mosquitoes, since it is possible that neither of the two men selected for the trial of mosquito transmission was susceptible to leprosy. It is highly probable that many individuals are not susceptible and that this accounts for the failure of the numerous previous negative inoculation experiments. It would appear that further experiments of this kind, using other biting insects and especially bedbugs, would be desirable, and, from the great importance of the subject, justifiable as well.

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ILLUSTRATIONS

TEXT FIGURES

INFLUENCE OF DENSITY OF POPULATION ON DEVELOPMENT OF LEPROSY

- FIG. 1. Chart showing average annual leper admission rate per thousand for each province. Provinces arranged in order of density of population.
2. Chart showing average annual leper admission rate per thousand. Provinces arranged in order of percentage of cultivated land.
 3. Chart showing leper rate charted on assumption that population lives on cultivated land.
 4. Chart showing leper rate charted on assumption that population lives on cultivated land and grassland.

INFLUENCE OF WEALTH ON DEVELOPMENT OF LEPROSY

5. Chart showing leper rates charted by provinces arranged in order of per capita annual income.
6. Chart showing leper rates charted by provinces. Provinces arranged in order of the ratio between density of population and per capita income.

THE EFFECT OF THE ADMINISTRATION OF ALCOHOL UPON THE RESULT OF THE WASSERMANN TEST IN YAWS MONKEYS

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In 1911 C. F. Craig and H. J. Nichols⁽¹⁾ published observations regarding the effect of the ingestion of alcohol on the result of the Wassermann test in syphilitic patients, and those observations have since been confirmed by many observers and are of great practical importance because they have demonstrated that negative Wassermann tests in individuals who have, within one or two days of the collection of blood, ingested considerable amounts of alcoholic liquors are not dependable from the diagnostic standpoint.

There are serologic analogies between syphilis and yaws. Yaws patients show a positive Wassermann reaction as frequently as do syphilitics. Philippine monkeys infected with yaws material also show a positive Wassermann reaction in the course of the disease as does man.^(2, 3)

The object of this investigation was to study further this serologic condition in experimental yaws monkeys.

PROCEDURE

I tested the Wassermann reaction in Philippine yaws monkeys before and after the administration of alcohol. At first alcohol was administered by mouth and under the skin at the same time, but as it was soon observed that the ingestion of alcohol by mouth or the injection of alcohol under the skin or into the muscles had, in every way, the same effect as to the result of the Wassermann test, the latter method was used in further experiments in order to simplify the procedure.

The experimental animals used in this investigation were Philippine monkeys infected experimentally with yaws strain Cadangan.⁽³⁾ The actual Wassermann tests were performed by the workers of the division of biology and serum laboratory, Bureau of Science, who are performing the regular routine

Wassermann tests in this institution and were unacquainted with the source of the blood samples submitted to them and consequently unprejudiced as to the interpretation of the findings. Table 1 shows the results.

As the table shows, either the ingestion of about 20 cubic centimeters of 48 per cent alcohol given by mouth or the parenteral administration of about 3 cubic centimeters of 95 per cent alcohol has rendered a double-plus positive Wassermann reaction negative; that is, incomplete hæmolysis became complete when the Wassermann reaction was repeated with a new blood sample obtained within from twenty-four to forty-eight hours after the administration of alcohol. This negative result, artificially induced, became usually positive again when the test was repeated on the same animal after a further interval of seventy-two hours; that is, within ninety-six hours after the administration of alcohol. The effect of the administration of alcohol on the result of the Wassermann reaction became noticeable as early as one hour after the administration of alcohol (see W-6). The change in the result of the reaction was so marked that a double-plus reaction, just before the administration of alcohol, became negative in twenty-four hours after the administration of alcohol, while a four-plus reaction (that is, complete inhibition of hæmolysis) became one plus (slight inhibition of hæmolysis). This effect continued sometimes for forty-eight hours or more.

This observation, therefore, has a practical significance as it demonstrates that a careful inquiry as to whether or not yaws patients have ingested, within one or two days, considerable amounts of alcoholic liquors should be made before the collection of blood for the Wassermann test is attempted. If it so happened, they should be instructed to discontinue the use of alcohol and report at a later date for the test.

CONCLUSIONS

1. The results of these experiments show that with regard to the effect of alcohol on the result of the Wassermann test yaws behaves the same way as syphilis.
2. The results bring further proof of the identity of the Wassermann reaction in Philippine yaws monkeys and in syphilitic patients.
3. The yaws-infected monkeys are suitable experimental animals for the study of the nature of the Wassermann reaction and for the study of the effect upon the Wassermann reaction of

TABLE 1.—*Showing the influence of the administration of alcohol on the result of the Wassermann test in yaws monkeys.*
 [++++, complete inhibition of hæmolysis; ++, incomplete inhibition of hæmolysis; +, slight inhibition of hæmolysis; ±, very slight inhibition of hæmolysis; —, complete hæmolysis.]

Designation of monkey.	Body weight.	Infection with yaws material.	First Wassermann test.	Dose of alcohol administered.			Result of Wassermann test.			
				By mouth, 48 per cent alcohol.	Under the skin, 95 per cent alcohol.	Into the muscle, 95 per cent alcohol.	Just before the administration of alcohol.	After the administration of alcohol.		
				cc.	cc.	cc.		Hrs.	Hrs.	Hrs.
W-6.....	2,850	Oct. 21, 1927	Feb. 9, 1928	20	4	—	++	1, ±	24, —	—
W-8.....	2,700	Oct. 25, 1927	Mar. 20, 1928	20	—	—	++	19, +	48, —	120, +
W-11.....	1,160	Nov. 7, 1927	Mar. 25, 1928	10	—	—	++	* 10, ±	—	—
T-15.....	2,100	Sept. 17, 1927	Mar. 25, 1928	—	5	—	++	48, —	72, ±	120, ±
W-16.....	2,570	Dec. 12, 1927	Mar. 26, 1928	—	3	—	±	24, ±	48, —	96, ±
W-5.....	1,300	{Oct. 18, 1927 Mar. 2, 1928}	Mar. 27, 1928	—	3	—	±	24, —	72, —	144, —
T-16.....	2,370	Sept. 20, 1927	Mar. 28, 1928	—	—	3	+	24, —	48, —	120, +
D-15.....	1,800	June 18, 1927	Mar. 28, 1928	—	—	2	+++	24, +	48, +	120, +++
F-31.....	2,500	Jan. 27, 1928	Mar. 28, 1928	—	—	4	+++	24, +++	48, +++	120, +++

* Died after the second collection of blood.

the administration of various drugs and chemicals as well as the effect of various conditions, physiologic and pathologic, upon this reaction.

I wish to express my sincere appreciation of the courtesies of Dr. Otto Schöbl, of the Bureau of Science, while this work was being done.

Thanks are due to Drs. Onofre Garcia and José Ramirez for their kindness in performing the Wassermann tests.

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TOTAL ALKALOIDS OF DATURA FASTUOSA LINNÆUS AND DATURA ALBA NEES FROM THE PHILIPPINES

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ONE TEXT FIGURE

In a recent paper on the pharmacognostical means for the identification of *Datura fastuosa* Linnæus and *D. alba* Nees, constituting the only representatives of *Datura* found in the Philippines, Santos⁽¹⁸⁾ has quoted the medicinal and poisonous properties attributed to them by other investigators. No quantitative chemical analysis, however, of the alkaloidal principle in *D. fastuosa* as grown locally has been reported since Bacon⁽²⁾ and Brill⁽³⁾ used the white-flowered *Datura*, which is the common one growing wild throughout the Philippines and found in waste places in and around Manila. Considering the fact that these two species of *Datura* are widely distributed in neighboring countries, the writer believes that it would be of interest to undertake a comparative study of the amount of total alkaloids in the different parts of these two plants when cultivated under conditions approximately identical.

The active constituents of the plants under the genus *Datura* belong to the so-called "solanaceous alkaloids," which are chiefly characterized by their mydriatic action. According to Henry⁽¹⁰⁾ this type of alkaloids includes the following:

1. Atropine and 2. Hyoscyamine, $C_{17}H_{23}O_3N$.
3. Nor-hyoscyamine, $C_{17}H_{21}O_3N$.
4. Meteloidine, $C_{13}H_{21}O_4N$.
5. Scopolamine (hyoscine), $C_{17}H_{21}O_4N$.
6. Apotropine and 7. Belladonnine, $C_{17}H_{21}O_2N$.
8. Pseudo-hyoscyamine, $C_{17}H_{23}O_3N$.
9. Tropacocaine, $C_{15}H_{19}O_2N$.

In the species of *Datura* so far investigated, Browne,⁽⁴⁾ Hesse,⁽¹¹⁾ Kircher,⁽¹³⁾ Andrews,⁽¹⁾ Pyman and Reynolds,⁽¹⁶⁾ Feldhaus,⁽⁸⁾ Carr and Reynold,⁽⁵⁾ and others have found the first five alkaloids above mentioned. The occurrence of more

than traces of atropine in solanaceous plants, however, is questioned by Henry,⁽¹⁰⁾ who contends that probably it may be the resultant product of the isomerization of hyoscyamine by the agents used during the process of extraction.

Hesse,⁽¹¹⁾ who investigated the Chinese species of *Datura alba*, states that the flowers contain scopolamine, hyoscyamine, and atropine; while Andrews⁽¹⁾ and Schmidt,⁽¹⁹⁾ who analyzed *D. metel*, which I believe is identical with the Philippine white-flowered *Datura*, have obtained only scopolamine and hyoscyamine from samples of leaves and seeds. Bacon,⁽²⁾ on the other hand, working on *D. fastuosa* Linnæus var. *alba* Clarke from the Philippines (*D. alba* Nees), found the same active constituents detected by Hesse,⁽¹¹⁾ as indicated above.

With respect to *D. fastuosa* Linnæus, so far the only report is that of Andrews,⁽¹⁾ who examined chemically *D. fastuosa* var. *niger* from Assam, India. He claims that the twigs and the leaves contain scopolamine and hyoscyamine, while the roots and the fruits contain only scopolamine.

EXPERIMENTAL

Preparation of samples.—The material for this investigation was obtained from the plants cultivated in the botanical garden of the University of the Philippines. Samples of leaves, flowers, fruits, stems, and roots were collected at intervals during 1927 in order that any variation of the alkaloidal content traceable to the different stages of growth could be detected. In the case of the fruits, the seeds were separated from the corresponding pericarp and other inner parts of the fruit. It was found that the color and the hardness of the seeds offer a fairly good basis for determining the various stages of maturity of the fruit. The different samples of fruits selected are therefore characterized as follows:

1. *Very young fruit.*—This represents the very early stage of the fruit, just after the caducous corolla has fallen off where the seeds are soft and whitish.
2. *Young fruit.*—An immature fruit with yellowish white seeds which can easily be crushed between the fingers. The color is very prominent along the border of the seed.
3. *Fairly mature fruit.*—This stage is characterized by having seeds that are yellow in the center and brownish around the

edges. The seed coat is well developed and can be punctured by the needle with difficulty.

4. *Mature fruit*.—The mature fruits are distinguished from the younger ones by having seeds that are yellowish brown, somewhat shriveled and hard. The pericarp is rather soft, spongy in texture, and easily disintegrated.

All the samples of the various parts of the plant were air-dried and reduced to fine powder. Moisture determination for each sample was then carried out in order to express the percentage of total alkaloid based on moisture-free samples.

Methods of analysis.—There are several methods that can be used for the quantitative determination of the solanaceous alkaloids. Consequently, preliminary experiments were performed to compare the methods proposed by Keller as modified by Schmidt,⁽¹²⁾ Dunstan and Ransom,⁽⁷⁾ and Andrews;⁽¹⁾ these three being selected because they have been employed by many investigators.

Table 1 shows that Keller's method modified by Schmidt gives lower results than the two other methods. This difference may be due to the fact that in Keller's method as modified by Schmidt the percentage of alkaloids is expressed in terms of a single alkaloid since the determination is volumetric. This has its drawback when the sample contains more than one alkaloid. As Andrews's method is applicable to all samples irrespective of their source, it was adopted except that continuous percolation with hot alcohol was used in order to shorten the time consumed for the extraction. As a whole, the method used is as follows:

A 15- to 20-gram sample is placed in a Soxhlet extraction apparatus and the percolation with hot alcohol is continued until the percolate is colorless and does not leave more than traces of solid matter. The alcoholic extract is distilled under diminished pressure until nearly all the solvent is recovered. The semi-solid residue is then treated with small quantities of warm water and, finally, with very dilute sulphuric acid to insure the complete extraction of the alkaloids. The combined aqueous acid liquid is then shaken with ether, made weakly alkaline with dilute ammonia, and shaken several times with chloroform to remove the alkaloids. The chloroform extract is washed with water in the separatory funnel, dried over anhydrous sodium sulphate, and then distilled under diminished pressure. The

total crude alkaloids thus obtained are dissolved in a small excess of very dilute sulphuric acid, and the acid solution is shaken with ether to remove traces of coloring matter and other impurities soluble in ether. This acidified aqueous liquid is then rendered slightly alkaline with dilute ammonia, and the alkaloids are extracted repeatedly, first by shaking with ether and then with chloroform. Both the ether and the chloroform extracts are washed with a little water, dried over fused anhydrous sodium sulphate, and after recovery of the solvents, the two fractions of alkaloids are dried in a vacuum desiccator and weighed. The combined weights of the two extracts give the "total alkaloids" contained in the sample.

TABLE 1.—Comparison of analytical results for total alkaloids from the seeds of *Datura alba* Nees.

Method.	Total alkaloids.		
	Sample 1.	Sample 2.	Mean.
	Per cent.	Per cent.	Per cent.
Keller modified by Schmidt.....	0.354	0.358	0.356
Dunstan and Ransom.....	0.373	0.370	0.372
Andrews.....	0.375	0.372	0.374

RESULTS

In Table 2, which represents the results of the investigation for the two plants, the various stages of the seeds and the pericarp correspond to the different ages of the fruit already described. The amount of alkaloids found in the pericarp also includes that from other inner parts of the fruit. Therefore, the total alkaloids present in a given stage of the fruit are computed by adding the percentages obtained from the seeds and the pericarp.

Leaves.—The leaves of *Datura fastuosa* contain slightly more alkaloids than those of *Datura alba*. In both plants the young leaves have a higher alkaloidal content than either the full-grown or the senescent leaves.

Flowers.—The results obtained from the flowers run parallel with those from the leaves, the young flower buds containing more alkaloids than either the unopened or the mature flowers; the flowers of *Datura alba*, however, contain more than those of *Datura fastuosa*.

Seeds.—As was to be expected, the mature seeds contain more alkaloids than the immature ones. This is true in both plants,

but as a whole the seeds of *Datura fastuosa*, except the very young ones, are higher in alkaloidal content than those of *Datura alba*.

Pericarp.—The results indicate that the pericarp of the very young fruit has more alkaloids than that of the other stages of the fruit analyzed. This is just the reverse of the condition found in the seed, for the high alkaloidal content of the mature seed is accompanied by the low percentage of the alkaloid in the corresponding pericarp, while the low percentage of total alkaloids in the very young seed is balanced by the high alkaloidal value of the very young pericarp. The pericarp of the fruit of *Datura fastuosa* yields more alkaloids than that of *Datura alba*.

Fruit.—The fruits of *Datura fastuosa* have a higher percentage of total alkaloids than the fruits of *Datura alba*. In both species, the very young and the mature fruits contain more alkaloids than the nearly mature fruits.

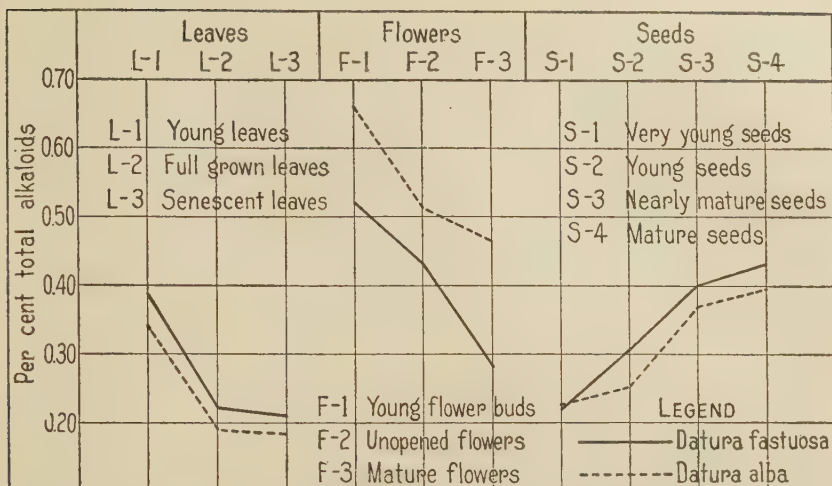
Stem and root.—There is only a very slight difference of total alkaloids found in the stems of *Datura fastuosa* and in those of *Datura alba*. However, the root of *Datura fastuosa* contains more alkaloids than that of *Datura alba*.

DISCUSSION OF RESULTS

The quantitative results, Table 2, show that the leaves, the seeds, the fruits, and the roots of *Datura fastuosa* have more alkaloids than the corresponding parts of *Datura alba*, while the flowers of the latter exhibit higher alkaloidal value than those of the former. The high percentage of alkaloids in the pericarp of the young fruit accompanied by the low amount of alkaloids in seeds of the same age, and the reverse condition in the mature fruit and seeds, indicates that during maturation of the fruit there is a migration of alkaloids from the pericarp to the seeds. There is only a very slight difference found in the alkaloidal content of the stems of the two species. When these results are compared with those of Table 3, it appears that the two species of *Datura* examined compare very favorably with the same or closely allied species of *Datura* grown in other countries. It can also be seen from Table 3 that my results for *Datura alba* lie within those obtained by Brill⁽³⁾ for the same plant. The fluctuation of alkaloidal values for any given part of the plant as indicated in Brill's analysis is due to the fact that the samples were dried under different conditions since the object was to show the effects of drying on the alkaloidal content.

TABLE 2.—Total alkaloids in various parts of *Datura fastuosa* Linnæus and *D. alba* Nees.

DATURA FASTUOSA.			
Part of plant.	Sample 1.	Sample 2.	Mean.
	Per cent.	Per cent.	Per cent.
Young leaves.....	0.385	0.391	0.388
Full-grown leaves.....	0.221	0.222	0.221
Senescent leaves.....	0.212	0.209	0.210
Young flower buds.....	0.522	0.520	0.521
Unopened flowers.....	0.441	0.440	0.440
Mature flowers.....	0.285	0.282	0.283
Very young seeds.....	0.224	0.225	0.224
Young seeds.....	0.304	0.312	0.308
Nearly mature seeds.....	0.400	0.401	0.400
Mature seeds.....	0.453	0.433	0.443
Very young pericarp.....	0.406	0.415	0.410
Young pericarp.....	0.238		0.238
Nearly mature pericarp.....	0.236	0.235	0.235
Mature pericarp.....	0.223	0.226	0.224
Very young fruit.....	0.630	0.640	0.635
Young fruit.....	0.542	0.550	0.546
Nearly mature fruit.....	0.636	0.636	0.636
Mature fruit.....	0.676	0.659	0.667
Young stem.....	0.217	0.220	0.218
Old stem.....	0.136	0.134	0.135
Root.....	0.252	0.244	0.248
DATURA ALBA.			
Young leaves.....	0.345	0.340	0.342
Full-grown leaves.....	0.191	0.190	0.190
Senescent leaves.....	0.183	0.180	0.182
Young flower buds.....	0.660	0.661	0.660
Unopened flowers.....	0.513	0.512	0.512
Mature flowers.....	0.460	0.462	0.461
Very young seeds.....	0.225	0.229	0.227
Young seeds.....	0.250	0.252	0.251
Nearly mature seeds.....	0.369	0.370	0.369
Mature seeds.....	0.392	0.394	0.393
Very young pericarp.....	0.347	0.347	0.347
Young pericarp.....	0.130	0.134	0.132
Nearly mature pericarp.....	0.076	0.077	0.076
Mature pericarp.....	0.061	0.066	0.063
Very young fruit.....	0.572	0.576	0.574
Young fruit.....	0.380	0.382	0.381
Nearly mature fruit.....	0.445	0.447	0.446
Mature fruit.....	0.453	0.460	0.456
Young stem.....	0.217	0.215	0.216
Old stem.....	0.151	0.156	0.153
Root.....	0.139	0.140	0.139

FIG. 1. Percentage of total alkaloids in some parts of *Datura fastuosa* and *Datura alba*.TABLE 3.—Alkaloidal content of *Datura fastuosa*, *Datura alba*, and *Datura metel*^a as reported by other investigators.

Species of <i>Datura</i> .	Part of plant.	Total alkaloids.	Investigator.
		Per cent.	
<i>Datura fastuosa</i> var. <i>niger</i> , from India.	Fruits.....	0.202	A. E. Andrews. (1)
	Leaves and branches...	0.119	
	Roots.....	0.101	
<i>Datura fastuosa</i> var. <i>flor. alb.</i> <i>plen.</i>	Seeds.....	0.223	E. Schmidt. (19)
	Leaves.....	0.25–0.55	
<i>Datura metel</i>	Fruits.....	0.12	A. E. Andrews. (1)
	Seeds.....	0.23–0.50	
	Roots.....	0.10–0.22	
	Leaves.....	0.210	
<i>Datura fastuosa</i> Linn. var. <i>alba</i> Clarke, from the Phil- ippines.	Seeds.....	0.465	R. F. Bacon. (2)
	Wood and roots.....	0.170	
	Green leaves.....	0.210–0.406	
	Stems.....	0.208–0.440	
	Flowers.....	0.205–0.489	
<i>Datura alba</i> Nees, from the Philippines.	Immature seeds.....	0.248	H. C. Brill. (3)
	Mature seeds.....	0.393–0.539	
	Immature fruit.....	0.131–0.409	
	Nearly mature fruit.....	0.325	
	Mature fruit.....	0.175–0.380	
	Immature pods.....	0.081	
	Mature pods.....	0.076–0.327	
<i>Datura alba</i> , from China.....	Flowers.....	0.485–0.550	F. Browne. (4) O. Hesse. (11)

^a *Datura metel* is included in this table since it is closely allied to, if not the same as, *Datura alba* Nees.

The consistent parallel variation observed in *Datura fastuosa* and *Datura alba* with respect to the alkaloidal constituent of their different parts varying in age, as shown in fig. 1, may have another significance on the mode of formation of alkaloids in plants. Thus, the presence of exceedingly high total alkaloids in the early stage of development of the leaves, the flowers, and the fruits suggests that alkaloidal synthesis in plants is undoubtedly related to the amount of nutrient materials present in the parts of the plant at different physiological periods; for taking into account the interrelationship of growth and vital activity, it seems very probable that the nature and the excess of food materials in different tissues and organs of the plant are influenced by the intensity of growth in the parts concerned. In this connection Gustafson's paper⁽⁹⁾ concerning growth studies on fruits is of interest. It is, therefore, significant to note that at first glance the low percentage of total alkaloids in the leaves, the flowers, and the stems at their maturity seems to be not in accord with Pictet's hypothesis⁽¹⁵⁾ on the formation of alkaloids containing a pyrrol ring or a pyridine nucleus. According to this theory, the mother substances of alkaloids are the nitrogenous decomposition products of more-complex substances, such as proteins and chlorophyll, as a result of metabolic processes. From this point of view, we should expect to find the reversed condition where the leaves, the flowers, and the fruits in their early stage of growth should have a much lower alkaloidal content than the mature ones. The writer's findings⁽¹⁴⁾ on the nitrogen partition of the leaves of *Oenothera pratincola*, in connection with the problem on disease resistance, is directly concerned in this matter. It was found that in the leaves of *Oenothera pratincola* where there is chlorophyll degradation, the proportion of simpler nitrogenous compounds, like amino acids, acid amides, and ammonia, to the total nitrogen is higher than in full-grown leaves where chlorophyll degeneration is not observed. This is in keeping with the observation that protein synthesis in the leaves decreases in the autumn due to the degradation of the chloroplasts.⁽²⁰⁾ On the other hand, it is possible to reconcile the results obtained from the two *Datura* plants with Pictet's hypothesis if we recall that Combes,⁽⁶⁾ Rippel,⁽¹⁷⁾ and others have confirmed the autumnal evacuation of nitrogen from the leaves to the branches. It may be then that the low alkaloidal content of the leaves and other parts when they are old is due to a considerable reduction of nitroge-

nous substances available for alkaloidal synthesis. As a whole this only means that purposeful experiments in this direction would yield results of lasting value in rendering possible a clear understanding of the physiological conditions governing the formation of alkaloids in plants.

SUMMARY AND CONCLUSIONS

1. This is a comparative study of the total alkaloids in various parts of *Datura fastuosa* Linnæus and *Datura alba* Nees from the Philippines. Incidentally, it also touches upon the variation of the alkaloidal content of the plant as exhibited by its various parts at different stages of growth.

2. Samples representing different ages of the leaves, the flowers, the fruits, and the stems were collected from plants of the two species of *Datura* grown side by side in the botanical garden of the University of the Philippines.

3. *Datura fastuosa* Linnæus contains more alkaloids in its leaves, seeds, fruits, and roots than does *Datura alba* Nees; the flowers of the latter have a higher percentage of total alkaloids than do those of the former.

4. As the fruit ripens there is a migration of alkaloids from the pericarp to the seeds.

5. In alkaloidal constituent *Datura fastuosa* Linnæus and *Datura alba* Nees from the Philippines compare very favorably with the same plants or closely allied species found in neighboring countries.

6. With the exception of the seeds, the young portions of the different parts of the plant yield more alkaloid than the corresponding mature portions.

7. The possible relation of the nutrient materials in the tissues and the organs of the plant at different physiological periods to the alkaloidal synthesis in plants is presented.

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ILLUSTRATION

TEXT FIGURE

FIG. 1. Chart showing the percentage of total alkaloids in some parts of
Datura alba and of *Datura fastuosa*.

AN ASPHALTITE FROM THE PHILIPPINE ISLANDS

By EDWIN T. HODGE

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During an investigation of the solid natural bitumens in Oregon ¹ a specimen from the Philippine Islands was analyzed with results that furnish additional data upon this material, making possible the determination of its specific character, the material from which it was derived, and its origin. The samples studied were collected and its occurrence described ² by Wallace E. Pratt, from the southwest slope of Mount Benao at the north end of Leyte Island.

The Leyte asphalt has been previously studied by Nelson ³ who summarizes his results as follows:

The natural solid bitumens occurring in Leyte Province have been classed as "pure bitumen" and bitumen-impregnated rock, and have been studied from a chemical and physical standpoint showing:

1. That Leyte pure bitumen differs from other mineralogical deposits of bitumens, such as ozocerite, montan wax, hatchettite, and scheererite, but is related to ozocerite. It is suggested that the pure bitumen of Leyte be termed leyteite.

2. That the chemical and physical properties of the bitumen found in the impregnated rock differ from those of the pure bitumen.

3. That the mineral aggregate of the bitumen-impregnated rock is principally limestone.

4. That the bitumen-impregnated rock would be suitable paving material, both in its natural state and after the introduction of fillers and fluxes.

CHEMICAL AND PHYSICAL PROPERTIES

The material occurs in large pure specimens. It is jet black, has conchoidal fracture and brilliant luster, and displays on paper and porcelain a dark brown streak. It is very brittle, so much so that when tested between the teeth it breaks up continuously into a fine black powder no fragments of which are maleable. When tested with a steel needle for penetration any pressure sufficient to mark it chips out small conchoidally fractured flakes.

¹ Hodge, E. T., Bull. Am. Assn. Petroleum Geologists 11 (April, 1927) No. 4.

² Philip. Journ. Sci. § A 10 (1915) 241.

³ Philip. Journ. Sci. 22 (1923) 617.

TABLE 1.—Analyses of grahamites and manjaks.

	Grahamite. ^a	Grahamite. ^b	Sample S. ^c	Philippine sample. ^d	Manjak. ^e	Untahite. ^f
Color.....	Jet black.	Jet black.	Jet black.	Jet black.	Jet black.	Black.
Luster.....	Brilliant.	Brilliant to dull.	Brilliant.	Brilliant.	Brilliant.	Brilliant.
Streak.....	Brown.	Brown.	Black.	Brown.	Brown.	Brown.
Fracture.....	Conchoidal to hackly.	Conchoidal to hackly.	Conchoidal.	Conchoidal.	Conchoidal to hackly.	Conchoidal.
Specific gravity.....	1.14-1.145.	1.07-1.08.	1.05-1.068.	1.029-1.058.	1.08-1.175.	1.01-1.71.
Hardness.....	2.	2.5.	2.0.	2.5.	2.	2-2.5.
Electrical charge.....	Positive.	Positive.		Positive.		Positive.
Softening point..... °C	175-315.	330.	150, imperfect.	360.	139-162.	125.
Fusion point..... °C	Initial 200, co- kes 175.	340.	Intumesces.		145-220.	Initial 200 to 205.
Volatile at 165 °C..... per cent.	0-1.	0-1.		0-1.		1.0.
Fixed carbon..... do	30-56.4.	23.	26-62.	55.	24.7-31.7.	10-35.
Ash..... do	30-56.4.	Spongy coke.	Delicate coke.	47.5 coke.	4.4-13.9.	
Soluble in carbon bisulphide (CS ₂)..... do	45-100.	75.	94.53.	98.5.	93.4-98.3.	98-100.
Soluble in carbon tetrachloride (CCl ₄)..... do	20-99.	53.		11.0.		99.6-100.
Carbenes..... do	0.80.	22.		87.5.		0-2.0.
Paraffin..... do			3.89.			
Soluble in benzene..... per cent.	Partly.	25.		50.0, solution dark brown.	84.3-95.3.	98-100.
Soluble in petroleum ether; boiling point, 40-60° C.; specific gravity, 633..... do	Trace-50.	0.		5.0.	22.2-36.4.	20-60.
Soluble in ethyl alcohol..... do	0.	0.		0.00.		0-54.6.
Soluble in chloroform..... do	90-100.	70.5.		100.0.		Partially.
Asphaltenes..... do		70.5.				

With sulphuric acid (H_2SO_4).....	Decomposes.....	Decomposes.....	Decomposes.....
With sodium hydroxide (NaOH).....	0.....	0.....	0.00.....
Order of gas.....			Petroleum.....

^a According to Kirkpatrick, Redwood, Danby, Abraham, Dana, Smith, Scoth, Eldridge, and Hodge.

^b Sample from Huntley Ranch near Clarno, Oregon, analysis by Hodge.

^c Philippine material, analysis and data by Pratt.

^d Philippine material, analysis by Hodge.

^e According to Kirkpatrick, Redwood, Danby, Abraham, Eldridge, and Hodge.

^f According to Kirkpatrick, Smith, Scott, Redwood, Danby, Abraham, Eldridge, and Hodge.

Fragments observed under the microscope are gray. When rubbed they are positively electrified; heated, they decrepitate, give off a gray gas with a faint petroleum odor, and small pieces finally melt down to a brilliant black fixed carbon. This button heated in a Bunsen flame intumesces and forms a gray bubble-filled slag. When heated in a crucible in a candle flame a sticky viscous liquid is obtained, which can be drawn out into long threads but does not stick to cold paper. The material melts at 360° C. to a jet black liquid, accompanied by a slight swelling and the production of a gray gas with a faint petroleum odor. Tested for fixed carbon and ash the material decrepitates and emits a gray gas with faint streaks of brown and a petroleum odor. Later the gas gets browner and has a faint ammonia smell. Finally only a gray gas is given off. The material does not melt down. It produces a brownish volatile stain of oil on the sides of the crucible. Fixed carbon is 55 per cent; greenish gray vesicular slag is 47.5 per cent.

Table 1 gives the properties of grahamite and manjak as tested and defined by many authorities.⁴ In this table these analyses are compared with an analysis of grahamite from Oregon⁵ and with the analyses of Philippine material. A comparison of these analyses with those of other native asphalts shows no similarity, and the comparison in this paper is, therefore, only with manjak and grahamite and with uintahite.

Richardson says that uintahite is derived from a nonparaffin oil and is not soluble in paraffin residues. The very small percentage of paraffin scales and the low solubility in benzine and, especially, in petroleum ether suggest that this sample has certain uintahite qualities. However, uintahite never has over 2 per cent of carbenes, and this sample contains 87.5 per cent. Grahamite, however, does not meet the above objection though it should not contain any paraffin scale. Grahamite in all re-

⁴ Herbert Abraham, *Asphalts and Allied Substances*. New York, D. Van Nostrand Co. (1918).—Arthur Danby, *Natural Rock Asphalts and Bitumens*. London, Constable, Ltd. (1913).—G. H. Eldridge, *The asphalt and bituminous rock deposits of the United States*, U. S. Geol. Surv. Ann. Rept., Part I (1901) 22; *The asphalt and bituminous rock deposits of the U. S.*, Bull. U. S. Geol. Surv. 213 (1902) 296–305; *Formation of asphalt veins*, Econ. Geol. 1 (1906) 437–444.—Sidney D. Kirkpatrick, *Marketing the natural hydrocarbons*, Eng. and Min. Journ. Press (February, 1925) 329.—Sir Boverton Redwood, *Treatise on Petroleum*. London, G. Griffin & Co. 1 (1922) 337.—Wilfred W. Scott, *Standard Methods of Chemical Analysis*. New York, D. Van Nostrand Co. (1917).

⁵ Hodge, loc. cit.

ported samples never has a specific gravity below 1.14. In color, luster, streak, and fracture our Philippine sample is identical with grahamite. The high fixed carbon and, according to Richardson, the fact that grahamite may be derived from paraffin-base oils further favors this conclusion. In Table 1 the Philippine material is shown to agree with grahamite in all respects except specific gravity, melting point, and solubility in carbon tetrachloride (CCl_4). Thus it agrees more closely with grahamite than with uintahite. Manjak is closely related to grahamite, and in part agrees with the Philippine sample, especially in specific gravity. The Philippine material lies between manjak and grahamite (see Table 2). Further evidence in support of this conclusion is given below.

TABLE 2.—*To show that the Philippine sample is between manjak and grahamite.*

	Carbon bisulphide (CS_2).	Carbon tetra- chloride (CCl_4).	Carbenes.	Petroleum ether.	Chloro- form.	Asphal- tenes.	Ash.
(a).....	45-100	20-99	0-80	4-50	90-100	90-100	30-56
(b).....	75	53	22	0	70.5	70.5	(*)
(c), (d).....	96	11	87.5	5.0	100.0	100.0	47.5
(e).....	93.98			22-36			4.13
(f).....	98-100	99.6-100	0-2.0	20-60	(b)		

^{a-f} See Table 1.

^g Spongy coke.

^h Partially.

The material analyzed by the author (sample d of Table 1) is the same as that reported by Pratt ⁶ (sample s). The sample was collected by Pratt and brought to the University of Oregon by Warren D. Smith, one time chief of the division of mines, Philippine Bureau of Science. The two analyses agree in all fundamental respects, indicating a high percentage of accuracy in both. Thus the fact that the sample of the first analysis intumesces suggests that it does not soften at 150° C. and that the volatile elements, if measured, would have been very low. The 55 per cent of the author's analysis approaches the average of 26 to 62 per cent of fixed carbons in Pratt's analysis. The discussion, therefore, in this paper will be based upon the latest analysis; that is, the author's.

GENESIS

The additional data herein presented confirm some and are at variance with other conclusions reached in Pratt's paper.

⁶ Loc. cit.

Thus the high percentage of carbenes⁷ confirms the view that the asphalts have been metamorphosed by heat. Pratt⁸ says:

It is believed that the intrusions have been instrumental in driving the petroleum upward through the surrounding rocks. One of the observed petroleum seeps is on the contact of an apparently intrusive holocrystalline rock in the Vigo shale. All the petroleum and solid bitumen occurrences are near intrusions, and viscous or semiliquid bitumen is found in concretions and brecciated concretionlike bodies, the origin of which may reasonably be attributed to solutions given off by buried intrusions.

In this respect the asphalts of the Philippines are similar to those in Oregon and dissimilar to many occurrences in other parts of the world.

The low specific gravity and the presence of paraffin scales of the material lead Pratt⁹ to state—

The Leyte natural bitumens are derived from a paraffin base petroleum. This fact at once distinguishes them from asphalt, as most commonly defined, which is derived either naturally or artificially from petroleum with an asphaltic base. The natural bitumen . . . was classified at the time of its discovery as gilsonite (or uintahite, another name for the same mineral) . . . Its specific gravity and its hardness are both less than the corresponding figures for gilsonite. Moreover, gilsonite, according to Richardson, . . . is derived from a nonparaffin oil and is not soluble in paraffin residues. All the Leyte bitumens are paraffin-bearing and are, therefore, not gilsonite according to Richardson. . . .

Grahamite, another natural bitumen, has something of the physical appearance. . . . But grahamite is heavier than any of the Leyte bitumens and has a higher proportion of fixed carbon.

Ozocerite is defined as a native paraffin, the physical appearance of which varies. Its color may be the same as that of some of the Leyte bitumens, its specific gravity is somewhat less, and it probably contains a higher average proportion of paraffin.

The difficulties stated above in attempting to determine the character of the Leyte material may be solved by a study of the new analysis. A low specific gravity in natural bitumen is usually accompanied by a decrease in ash and an increase in petrolenes.¹⁰ The ash in the Leyte sample, however, is rather high, which assuming the sample to be pure and free of contaminated mineral fragments is due to oxidized hydrocarbons. The high ash, therefore, might be explained by its long surface

⁷ The term "carbene" is employed for those constituents soluble in carbon bisulphide but not soluble in carbon tetrachloride. Carbenes are especially common in grahamite, native asphalts, and asphaltites.

⁸ Philip. Journ. Sci. § A 10 (1915) 253.

⁹ Op. cit. 263.

¹⁰ Petrolenes are the volatile hydrocarbons of petroleum ether, ethyl ether, or acetone.

exposure in a tropical country expedited by the heat of adjacent igneous intrusions.

The next question now becomes, What material has been oxidized to produce the high ash? We note that the low specific gravity is not accompanied by high petrolenes. These are close to the minimum for grahamite and very low for manjak. If the original components had undergone much inspissation or molecular condensation there should be more than 5 per cent of petrolenes present. The low petrolenes might be interpreted to indicate that the original material was rich in grahamite and low in manjak. This conclusion is not in harmony with other evidence. It were better to decide that the compounds giving rise to the high ash were not those rich in petrolenes but asphaltenes¹¹ which constitute 100 per cent. The high fusibility shows that these asphaltenes have been altered. Here we have an explanation of the high ash.

The Leyte specimen is apparently a substance that has been oxidized by exposure and metamorphosed by heat without any great molecular condensation of the petrolenes or asphaltenes, because the material is insoluble in carbon bisulphide. The percentage of fixed carbon plus ash, which is very high for most grahamites or manjaks, is not due to pyrobitumens or asphaltic pyrobitumens.

All possible sources of the high ash and fixed carbon have now been considered, leaving only oxidation and thermal metamorphism as a possible explanation. The material has been altered in such a way as not to increase the specific gravity, but greatly to increase the melting point and the percentage of fixed carbon. The process may have been favored by the slow seepage of the Leyte asphalts to the surface.

Richardson states that when crude petroleum, which contains a high percentage of hydrogen, and therefore simple hydrocarbons, is subjected to heat and pressure under favorable conditions, a large volume of gaseous hydrogen is eliminated, resulting in the conversion of the hydrocarbons into compounds more complex both chemically and physically. Colloidal clay accelerates the process by acting as a catalyzer. The petroleum is converted into asphalt by emulsification with clay, sand, and water by means of natural gas at high pressure. Nutting¹² has found that petroleum and water are slightly soluble in each

¹¹ Asphaltenes (Danby) or malthenes (Richardson) are those parts dissolved by boiling turpentine or chloroform.

¹² Nutting, P. G., *Econ. Geol.* 21 (1926) 234-242.

other and both are strongly absorbed by silica. In the presence of this catalyzer the oil in the presence of water will not only be driven out of sands but will undergo a chemical change. This change may result in conversion into compounds of high fusibility but of low specific gravity. As the results of studies of the famous pitch lake in Trinidad, British West Indies, he concludes that the petroleum is converted into asphalt by emulsification with clay, sand, and water by means of natural gas at high pressure. Maltha and other soft asphalts, hard asphalt, the asphaltites (gilsonite and grahamite), the asphaltic pyrobitumens (elaterite and wurtzilite), and asphaltic pyrobituminous shales are probably successive stages in the metamorphosis of asphaltic petroleum. If the petroleum is of paraffin base, ozokerite and other mineral waxes are sometimes formed.

Finally, the evidence does not point to a paraffin-base oil as the source of the material. Richardson says that grahamite is due to a condensation of paraffin oils and so differs from uintahite and manjak, which are formed by unsaturated hydrocarbons. The formation of asphalt, related bitumens, and pyrobitumens presupposes the existence of petroleum containing asphaltic hydrocarbons, and that of ozokerite presupposes the existence of paraffinaceous petroleum.¹³

Pratt found 3.89 per cent of paraffin in the Leyte material. This small amount is in agreement with the 5 per cent of petroleues. Both these facts indicate that some grahamite is present but only in a small amount, perhaps to 10 per cent. In the main the material is manjak. Since the time of the Pratt paper our views have changed regarding the bases of petroleum. Thus, at present, an asphaltic base means a large amount of sulphur, and a paraffin base a low percentage of sulphur. In this paper "asphaltic" is used in the sense of being rich in cyclic compounds.

OTHER OCCURRENCES

Grahamites and manjaks are widely distributed. Grahamite has been found to be more widely distributed in the United States than most of the natural asphaltic hydrocarbons, notably in Richie County, West Virginia, and Payette and Stephens Counties, Oklahoma. The largest known vein, 19 to 25 feet wide and more than a mile long, is in Jackford Valley, near Tusahoma, Oklahoma. Unlike uintahite, it is found in several foreign countries; the deposits in Cuba, Mexico, and Trinidad Island are the most important. Other occurrences are in Utah,

¹³ Mineral Resources of the U. S. pt. 2 (1918) 467.

Colorado, and Oregon. The grahamite vein in West Virginia occupies a vertical fissure in the Waynesburg sandstone and adjoining beds above and below, all in the upper Carboniferous.¹⁴

The grahamite of Middle Park, Colorado, occurs in a vein that is considered to have been filled with liquid or viscous bitumen derived from adjacent or nearby strata.

Grahamite characteristically contains a large proportion of inorganic impurities. Ladoo¹⁵ says that "the Oklahoma deposit has been worked extensively and many thousand tons removed." Grahamite finds its chief use in the roofing industry, since when mixed with asphaltic fluxes it forms a rubbery, elastic covering very satisfactory for this purpose. With other bitumens it yields varnishes, rubber substitute for waterproofing, and compounding materials for electrical insulation. No commercial production of grahamite has been reported since 1924.¹⁶

Manjak¹⁷ is a term that originated on Barbados Island, where it was applied to a high-grade black bitumen, which breaks with a conchoidal fracture, exposing a bright, lustrous surface. Barbados manjak has a specific gravity of 1.10, a hardness of 2, and a fusion point of 320 to 430° F. It contains a varying amount of mineral matter and about 90 per cent of carbon, of which 25 to 30 per cent is fixed carbon. The essential characteristics of Barbados manjak are given in Table 3. Trinidad manjak differs principally from the Barbados in that it has a higher specific gravity (1.175) and a lower total carbon content. Both are exported to the United States, but the Barbados variety commands a much higher price. The following paragraphs and Table 3 are from Kirkpatrick's article, cited above.

Manjak finds its principal application in paints and varnishes, in which it is usually mixed with mineral oils. These are used as protective coverings on pipe lines, structural steel work, and machinery. An interesting application of some promise is as a pipe cement and packing at the joints of rotary drill pipe, especially in the oil fields.

According to recent quotations (January, 1925) Barbados manjak in one to five-ton lots sold in New York for the following prices: Grade A, 6c. per pound; Grade AA, fine 8c. per pound; and Grade C, lump, 12c. per pound.

¹⁴ Reports of Department of Interior, Geological Survey, House Documents 28: 228-229; 327-328.

¹⁵ U. S. Bur. Mines, Report of Investigations (May, 1920).

¹⁶ Hopkins, G. R., Asphalt and Related Bitumens in 1926, Dept. of Commerce, Bureau of Mines publication.

¹⁷ Kirkpatrick, S. D., Engineering and Mining Journal-Press 119 (1925) 331.

TABLE 3.—*Characteristics of Barbados manjak.*

	Grade—		
	A	AA	C
Total carbonaceous matter.....per cent..	86.10	95.58	98.37
Mineral matter (ash).....do..	13.90	4.42	1.63
Volatile matter on ignition.....do..	56.90	63.85	68.47
Fixed carbon on ignition.....do..	29.20	31.73	29.90
Solubility in petroleum ether.....do..	29.75	33.45	36.37
Solubility in 90 per cent benzol.....do..	84.30	94.48	92.23
Solubility in carbon disulphite.....do..	93.40	94.95	95.76
Specific gravity.....	1.146	1.153	1.128
Softening point (Kraemer and Sarnow).....°C..	162	160	139
Melting point (Kraemer and Sarnow).....°C..	180	177	145

THE BUMBLEBEES OF THE PHILIPPINE ISLANDS (BREMIDÆ: HYMENOPTERA)

By THEODORE H. FRISON

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ONE PLATE

In 1925, in the Philippine Journal of Science, I published a paper on the bumblebees of the Philippine Islands giving a summary of the systematic knowledge of the species and varieties and certain other information. The accumulation of additional material and exact information regarding the structural characters of the type (worker) of *Bremus irisanensis* (Cockerell) makes the publication of some supplementary notes and new keys highly desirable.

For additional material I am greatly indebted to Dr. H. Hedicke, of Berlin, Germany, who graciously sent me specimens of this genus from the Philippine Islands (including the male type of *B. imuganensis* Hedicke) from his collection. Several specimens were submitted, also, by the Hawaiian Sugar Planters' Association. To Dr. James Waterston, of the British Museum, and Mr. O. W. Richards, of Oxford, England, I am much indebted for critical notes regarding the structural characters of the worker type of *B. irisanensis* (Cockerell) and for comparing with the type two specimens submitted to them.

For the sake of convenience and clarity I have arranged my remarks in this paper under the following headings: Synonymy, descriptions, keys to species and castes, tabulation of descriptions, and bibliography.

SYNONYMY

BREMUS (HORTOBOMBUS) IRISANENSIS (Cockerell).

Bombus irisanensis COCKERELL, Ann. & Mag. Nat. Hist. VIII 5 (1910) 416, ♀.

Bombus irisanensis COCKERELL, Philip. Journ. Sci. 14 (1919) 81.

Bremus irisanensis FRISON, Philip. Journ. Sci. 27 1925) 116, ♀
[= ♀ not ♀].

Bremus irisanensis HEDICKE, Deutsch. Ent. Zeitschr. (1926) 421, ♀,
♂, and ♂.

The study of additional material soon after the appearance of my first paper (1925) on the bumblebees of the Philippine Islands revealed that two species of these large social bees were masquerading as a single species under the guise of similar colors and a similar color pattern. This was evident, likewise, from the illuminating remarks published by Hedicke in a recent paper (1926). Correspondence with Doctor Hedicke and the study of his specimens removed all doubt about the matter, but did not definitely establish which of the two species was entitled to the name of *irisanensis* (Cockerell).

As mentioned in the introduction of this paper, Doctor Watterston and Mr. Richards, of England, very kindly answered certain questions for me regarding the structural characters of the worker type of *B. irisanensis* (Cockerell). It is evident from their report that the type of *B. irisanensis* has a long malar space and that the metatarsus of the middle leg at its apical outer angle has a sharp spinelike projection. This is conclusive evidence that this species belongs to the section *Odontobombus* Kruger. A study of a male of this species establishes, also, that it belongs to the subgenus *Hortobombus* Vogt.

A study of a male, a queen, and workers of the species studied and recorded by Hedicke (1926) has established that his specimens are the true *irisanensis* as listed by him. Also, the females redescribed in more detail by myself (1925) are *irisanensis* as originally listed. They are, however, workers instead of queens. This is evident from a study of a queen submitted by Doctor Hedicke, which as he (1926) states measures 27 millimeters in length and has a spread of wings of 54 millimeters.

Since the male and the queen have never been described in detail, this is done in another part of this paper.

BREMUS (PRATOBOMBUS) BAGUIONENSIS (Cockerell).

Bombus irisanensis var. *baguionensis* COCKERELL, Philip. Journ. Sci. 16 (1920) 631, ♀.

Bremus irisanensis var. *baguionensis* FRISON, Philip. Journ. Sci. 27 (1925) 118, ♂.

The determination of the sectional and the subgeneric status of the true *irisanensis* (Cockerell) has made it necessary that the form described as *baguionensis* (Cockerell) be recognized as a valid species instead of a color variety. Specimens of *baguionensis* in my collection from the locality of the type and compared with the worker type by C. F. Baker have been available for this study, and the results have led to this conclusion.

Bremus baguionensis is a smaller species, with a malar space of moderate length, and in the females the metatarsus of the middle leg at its apical outer angle does not have a sharp spine-like projection. The genitalia of the male (Frison, 1925) affords final and conclusive proof that *baguionensis* belongs to the section *Anodontobombus* Krüger and to the subgenus *Pratobombus* Vogt.

BREMUS (PRATOBOMBUS) BAGUIONENSIS var. IMUGANENSIS (Hedicke).

Bombus imuganensis HEDICKE, Deutsch. Ent. Zeitschr. (1926) 422, ♂.

Bombus irisanensis FRISON, Philip. Journ. Sci. 17 (1925) 116, ♂.

The male described as *imuganensis* by Hedicke (1926) does not differ structurally from the male of *baguionensis*, as evidenced by the statement of Hedicke that "stimmt der Kopulationsapparat völlig mit Frison's Abbildung überein." Furthermore, a critical study of the type submitted by Hedicke bears out this contention.

In color *imuganensis* is essentially like *baguionensis*, except that the dorsum of the thorax is somewhat darker because of a slight admixture of dark hairs, and the fifth and sixth dorsal abdominal segments are black. Since the typical *baguionensis* has light ochraceous or light ferruginous pubescence on the fifth and sixth dorsal abdominal segments, the name *imuganensis* may be preserved as a varietal name for a black-tailed color variety of the species *baguionensis*.

Females referable to the color variety *imuganensis* Hedicke exist, as proved by the presence of such a female in my collection. Since the queen of *baguionensis* or its variety *imuganensis* has not been described, and many of the structural characters of the species were omitted in the original description, more-detailed descriptions of them are presented.

DESCRIPTIONS

Since the castes of bumblebees (queen, worker, and male) often differ in color from one another, as well as always in structure in the two sexes, I believe that detailed descriptions of them in addition to a detailed description of the type or holotype (whatever caste that may be) are advisable. Accordingly, under this heading I present the descriptions of several forms that have never been described. The preservation of actual single specimens of specimens used in preparing such descriptions is highly desirable and is aided by assigning to such specimens the frequently used terms "allotype" and "mor-

phototype." The term "allotype" is here used to designate the first described specimen of opposite sex to the type, whatever sex that may be, of a previously known species. The term "morphotype" is used for the second form of a dimorphic sex, which in the case of the bumblebees is always of the female sex (queen or worker). Naturally, here again the designation is applied solely to a specimen used in preparing the first description of the undescribed caste. Such designations as allotype and morphotype have no nomenclatorial significance unless they happen also to be at the same time paratypes. Even if they happen to be true paratypes, as is not the case with any of those designated in this paper, they have no more nomenclatorial significance than any other paratype.

BREMUS MEARNSI var. **BAKERI** (Cockerell).

Worker.—Pubescence on head entirely dark. Labrum with tuberclelike areas, which are prominent, shagreened on inner margins and shiny on outer margins, with a few large punctures on prominent elevated ridge, the space between tubercles nearly equal to the length of the second flagellar segment; shelflike projection broad, rugose. Mandibles distinctly three-toothed with slight indications of a fourth tooth at lower apical angles; setæ on distal portion of mandible between the raised longitudinal ridges very short, dense, golden in color. Clypeus smooth, shiny, with a few small punctures on its disk, and large punctures mixed with smaller ones on the lateral, dorsal, and anterior portions. Malar space slightly longer than its width at articulation of mandible (from precoila to postcoila), about one-half as long as the greatest width of, and one-fifth as long as, the compound eye; smooth, shining, but feebly punctate. Ocelli situated just above the narrowest part of the vertex; each lateral ocellus about its diameter distant from the median ocellus; area between lateral ocelli and median margin of compound eyes polished, almost impunctate. Flagellum slightly more than twice as long as the scape; third flagellar segment longer than the fifth, the fifth slightly longer than the fourth.

Dorsum and sides of thorax entirely with dark pubescence; center of disk smooth and impunctate.

Abdomen with the first four dorsal abdominal segments covered almost entirely with moderately long dark pubescence; that on fifth and sixth apical segments yellowish brown. Venter with light yellowish brown pubescence. Hypopygium without a median carina.

Legs with cuticle reddish brown; pubescence on trochanters light, on femora dark, on tibiæ and tarsi bright golden color. Metabasitarsus distinctly arcuate and outer surface nearly flat. Distal end of mesobasitarsus without a pronounced apical projection. Corbiculæ slightly shagreened.

Proximal portion of the wings yellowish, distal portion distinctly suffused, nervures prominent.

Length, 14 millimeters; spread of forewings, 31 millimeters; width of abdomen at second segment, 8 millimeters.

Morphotypic worker, Mount Canlaon, Occidental Negros, Philippine Islands, August 29, 1921, 5,000 feet elevation; collected by F. X. Williams. Deposited in the collection of the author. Another worker with the same data deposited in the collection of the Hawaiian Sugar Planters' Association. In general, the workers of this variety are replicas of the queens.

BREMUS IRISANENSIS (Cockerell).

Male.—Clypeus densely covered with long yellowish pubescence; that on occipital orbits, surrounding bases of antennæ and occiput, a black color. Malar much longer than its width at articulation of mandible between precoila and postcoila, about three-fourths as long as greatest width of and about one-third as long as length of compound eye. Ocelli very small, situated above narrowest part of the vertex, about their diameter removed from median ocellus. Space between each lateral ocellus and compound eyes smooth and shiny on mesal half and with large scattered punctures on outer half. Flagellum about four times as long as scape; third and fourth segments about equal in length, fifth much longer than either, middle flagellar segments distinctly arcuate.

Dorsum and upper anterior corners of pleura of thorax entirely black; mesopleura with yellow or fulvous-yellow pubescence. A narrow elongate impunctate area, somewhat shagreened, on disk of mesonotum.

Abdomen with yellow or fulvous-yellow pubescence on the first two dorsal abdominal segments, pubescence on the remaining segments black. Venter with blackish pubescence.

Sagittæ without a cyclelike hook at end; head widened and toothed on outer margin, terminating in a point; shaft with a pronounced downward directed tooth in middle of its length on lower margin. Uncus narrow and pointed at apex. Squama large, with a curved hooklike process on its mesal posterior margin. Vosellæ extending well beyond squamæ and considerably

inwardly; apex with a small hook directed outwardly. Dorsal view of genitalia shown in fig. 1. Inner and outer spathæ shown, respectively, in figs. 2 and 3.

Legs with cuticle blackish, with mostly black hairs except upon tarsal segments which are more golden brown. Hind tibiæ on outer surface convex, with numerous setæ bearing punctures. Metatarsus of middle leg at apical outer angle with a pronounced, sharp, spinelike projection. Metatarsus of hind leg concave, with hind margin somewhat arcuate; three and one-half times as long as greatest width.

Wings fuliginous throughout.

Length, 22 millimeters; spread of forewings, 47 millimeters; width of abdomen at second segment, 10 millimeters.

Allotypic male, Imugan, Luzon, Philippine Islands, 1923. Deposited in the collection of the author by permission of Doctor Hedicke, who first recorded but did not fully describe this male.

Queen.—Similar, except in size, in structural details, and in color characters, to the workers as redescribed by Frison (1925, pp. 116–117). In addition to that description the narrow elongate bare area on disk of mesonotum is somewhat shagreened; metatarsus of middle leg and hind legs at apical outer angles with pronounced, sharp, spinelike projections.

Length, 27 millimeters; spread of wings, 54 millimeters; width of abdomen at second segment, 11 millimeters.

Morphotypic queen, Imugan, Luzon, Philippine Islands, 1923. Deposited in the collection of the author by permission of Doctor Hedicke, who first recorded but did not fully describe this queen.

The additional characters here recorded for the queen of the typical *irisanensis* (Cockerell) are also found on the workers. In a previous paper in the Philippine Journal of Science (1925) I described two rather large females of *Bremus irisanensis* (Cockerell) and designated them as morphotypic queens, thinking them to be the queens of this species. The study of material submitted by Doctor Hedicke proves them to be large workers, and since these specimens have no nomenclatorial status they are to be disregarded as morphotypical specimens. The true queen is here described from a specimen which was a part of the original Hedicke material (1926; first authentic report of queen and male) and is now designated as the true morphotype. Likewise, the male from the same material is designated as the allotype.

BREMUS BAGUIONENSIS var. **IMUGANENSIS** (Hedicke).

Queen.—Face, occipital orbits, and occiput with black hairs. Labrum with tuberclelike areas moderately separated, lateral portions smooth and shiny, mesal portions with some large punctures and somewhat shagreened; shelflike projection rugose, about one-half as long as width of labrum, with its anterior margin strongly crescentic. Mandible three-toothed, with but a slight suggestion of a fourth tooth at lower apical angle. Clypeus with a smooth shining mesal area, punctate on lateral and upper portions. Malar space about as long as its width at articulation with mandible; about one-half as long as greatest width of and slightly less than one-fourth length of compound eye. Ocelli large, situated just above narrowest part of the vertex, just below supraorbital line; lateral ocelli slightly closer to the inner margin of the eyes than to one another; area directly lateral of each lateral ocellus mostly impunctate and shining, a few small punctures near margin of eye. Flagellum slightly less than twice as long as the scape; third antennal segment much longer than fifth, the fifth longer than the fourth.

Dorsum of thorax and upper anterior corners of pleura with black pubescence, that on remaining areas of pleura dark yellowish; a narrow, elongate, impunctate, smooth and shiny area in the center of disk.

Abdomen with dark yellowish pubescence on the first two dorsal abdominal segments, pubescence on remaining segments black. Venter with dark pubescence. Hypopygium without a median carina.

Legs with cuticle mostly black, but in places inclined to be reddish, particularly on hind corbiculae and tibiae. Corbicular fringes dark. Apical outer angle of metatarsus of middle leg with a pronounced, sharp, spinelike projection.

Proximal portion of the wings yellowish, distal portion distinctly suffused, nervures prominent.

Length, 18 millimeters; spread of forewings, 39 millimeters; width of abdomen at second segment, 8 millimeters.

Morphotypic queen, Mount Banahao, Tayabas Province, Luzon, Philippine Islands, April 29, 1921, 4,000 feet elevation; collected by F. X. Williams. Deposited in the collection of the author.

Superficially identical in coloration with *irisanensis* (Cockerell), but differs markedly in structural characters as pointed out in the key to females.

Keys to the Philippine species and varieties of *Bremus*.

QUEENS AND WORKERS (FEMALES)

1. Metatarsus of middle leg at apical outer angle with a pronounced, sharp, spinelike projection; ocelli small, each lateral ocellus nearly twice as far removed from median ocellus as its diameter; outer half of area between lateral ocelli and median margin of compound eyes strongly punctate; first and second dorsal abdominal segments with yellow or fulvous-yellow pubescence; very large queen (26 millimeters in length) and workers (15 to 20 millimeters in length).

Bremus (Hortobombus) irisanensis (Cockerell).

- Metatarsus of middle leg at apical outer angle without a pronounced, sharp, spinelike projection; ocelli large, each lateral ocellus less or not more than its diameter removed from median ocellus; outer half of area between lateral ocelli and median margin of compound eyes smooth, shiny, with but few small punctures; small queens (19 millimeters in length) and workers (11 to 13 millimeters in length) 2.
2. First and second dorsal abdominal segments with black or chocolate-brown pubescence 3.
 - First and second dorsal abdominal segments with yellow or fulvous-yellow pubescence 4.
 3. Pubescence of the thorax and basal, dorsal, abdominal segments nearly black; only the last two apical, dorsal, abdominal segments entirely reddish fulvous *Bremus mearnsi* var. *bakeri* (Cockerell).
 - Pubescence of the thorax and basal, dorsal, abdominal segments chocolate brown; last three apical, dorsal, abdominal segments entirely or nearly yellowish brown or fulvous *Bremus mearnsi* (Ashmead).
 4. Apical segments entirely or mostly black.

Bremus (Pratobombus) baguionensis var. *imuganensis* (Hedicke).

Apical segments with yellowish or golden brown pubescence.

Bremus (Pratobombus) baguionensis (Cockerell).

MALES

[The male of *Bremus mearnsi*, or of its variety *bakeri*, is not known.]

1. Metatarsus of middle leg at apical outer angle with a pronounced, sharp, spinelike projection; hind tibiæ on outer surface without a large more or less bare, impunctate area on middle portion; head of shafts of sagittæ of genitalia without a cyclelike hook.

Bremus (Hortobombus) irisanensis (Cockerell).

- Metatarsus of middle leg at apical outer angle without a pronounced, sharp, spinelike projection; hind tibiæ on outer surface with a more or less bare, shiny, impunctate area on middle portion; head of shafts of sagittæ of genitalia with a cyclelike hook (*Pratobombus*) 2.
2. Apical dorsal segments of abdomen with a considerable amount of yellow or fulvous-yellow pubescence.

Bremus baguionensis (Cockerell).

Apical dorsal segments of abdomen entirely dark or with fulvous-yellow pubescence restricted to last segment.

Bremus baguionensis var. *imuganensis* (Hedicke).

TABULATION OF CASTE DESCRIPTIONS OF SPECIES AND VARIETIES

Bremus mearnsi (Ashmead); type, worker (1905); collection United States National Museum.

Bremus mearnsi (Ashmead); morphotype, queen (Frison, 1925); collection United States National Museum.

Bremus mearnsi var. *bakeri* (Cockerell); queen (1920); collection Baker 11839.

Bremus mearnsi var. *bakeri* (Cockerell); morphotype, worker (Frison, 1928); collection Frison.

Bremus irisanensis (Cockerell); type, worker (1910) collection British Museum.

Bremus irisanensis (Cockerell); allotype, male (Frison, 1928); collection Frison.

Bremus irisanensis (Cockerell); morphotype, queen (Frison, 1928); collection Frison.

Bremus baguionensis (Cockerell); type, worker (1920); collection Baker 11841.

Bremus baguionensis (Cockerell); allotype, male (Frison, 1925); collection Frison.

Bremus baguionensis var. *imuganensis* (Hedicke); type, male (1926); collection Frison.

Bremus baguionensis var. *imuganensis* (Hedicke); morphotype, queen (Frison, 1928); collection Frison.

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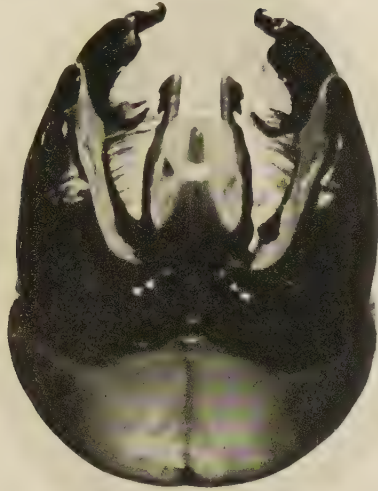
ILLUSTRATION

PLATE 1. *BREMUS IRISANENSIS* (COCKERELL)

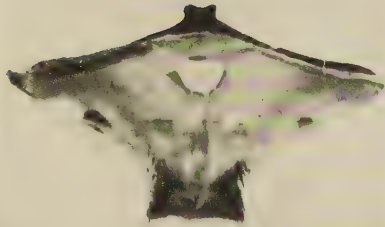
FIG. 1. Male genitalia.

2. Inner spatha.

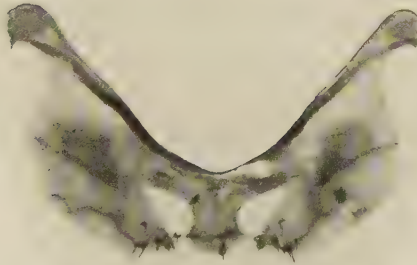
3. Outer spatha.



1



2



3

PLATE 1. BREMUS IRISANENSIS (COCKERELL).

NEW PHILIPPINE SHIPWORMS

By P. B. SIVICKIS

Of the University of the Philippines, Manila

THREE PLATES

Bartsch (1927) lists sixteen species of shipworms from the Philippines, four of which may be considered as of doubtful validity. In this paper I present fifteen species of these animals, none of which has been described before. Bartsch's specimens were collected from the deeper waters, while mine came from the shore, from floating wood, or from sand. None of them came from water deeper than one meter.

The classification here adapted is that of Bartsch as given in his monographs. It is based almost entirely on the shells and the pallets. As pointed out by Miller (1922) classification based on either shell or pallets alone is of doubtful value and can be resorted to only in cases of absolute necessity. For this reason I have left incomplete specimens for further study when more material may come to hand. The nomenclature of parts adopted by Bartsch and by Miller is used.

All three genera of teredos are represented in the Philippines. Future study may necessitate the creation of new genera. I am inclined to think that the shell described by Bartsch in *Eoteredo philippinensis* belongs to *Bactronophorus* and that the new generic name will not be applicable. On the other hand, it is possible that *Teredo hydei* and *T. apendiculata*, described in this paper, should be referred to a new genus. I admire Bartsch's creation of subgenera, but I cannot follow his example in this respect. I think that this group of animals is much larger than is generally supposed, at least in the Philippines, and our knowledge is still inadequate for the division into subgenera.

The type specimens of the species described herein have been deposited in the Bureau of Science, Manila.

Genus BANKIA Gray, 1840

The blade of the pallet consists of a series of cones which are free at their distal end. The cones may fit very closely

into each other or they may be open and end in spiny projections.

BANKIA TRIANGULARIS sp. nov. Plate 1, fig. 1.

Shell large and triangular; rather thin for its size. Sinus is in the form of an arc of an ellipse and runs along the dorsal border of the anterior part. Anterior part considerably wider than high (5 : 9). Both anterior and anterior median parts very finely ridged. The ridges are so fine that it is very difficult to count them even if magnified with a hand lens. A little over three hundred ridges were counted. Anterior median part very wide, about 1.5 times the rest of the median and the auricle combined. Middle median slightly declined posteriorly. Posterior median very narrow, just a little wider than middle median. Auricle very small or lacking. Line of junction between right and left shells very long, of the same length as distance from umbone to knob. The shell has a general appearance of a right triangle.

Inside of shell bluish white. The shelf is entirely at the rim of the posterior median until it reaches the auricle; from this point it merges with the auricle. The blade springs from the posterior median and the ventral border of the shell; usually it is small but in some cases about one-fourth of it may reach the anterior median part.

Pallets of medium size, with very closely packed cones. The composite nature of the pallets is seen best from the outer surface.

The specimens were collected in Cebu by R. S. Filoteo.

BANKIA ORYZAFORMIS sp. nov. Plate 1, fig. 2.

Shell small, thin, transparent, and light blue. The anterior part spreads fanlike. Width and height of anterior part about equal. Ridges on anterior median finer than those of anterior part, but in spite of inequality the number is about the same. Middle median narrow. Posterior median about equal to width of middle and posterior medians combined. Irregular growth lines well marked. Auricle rather small, at a slight angle with posterior median. In the inside of the shell the junction between the auricle and the posterior part is marked by a strong shelf. Blade short and broad; it springs from the rim of the callus. Knob prominent.

Pallets of loose cone-in-cone type. Margins of cones entirely free and smooth. Peduncle thin and even in thickness throughout, except near the blade where it becomes abruptly thickened.

The specimens were found in a piece of wood (*Xylocarpus* sp.) exposed during low tide at Puerto Princesa, Palawan.

BANKIA QUADRANGULARIS sp. nov. Plate 1, fig. 3.

Shell above medium in size and thickness. The usual callus extends in a straight line along the rim of the anterior part. This gives it the shape of a more or less irregular quadrangle. Anterior part wider than high (7 : 5). Ridges fine but distinct; about ninety can be counted. Denticles very fine. Anterior median slightly wider than middle and posterior medians combined. Ridges of anterior median similar to those of anterior part; they coördinate at the junction of the two parts. Middle median part narrow, but well marked on both sides. Posterior median well arched and merges into a small auricle.

Inside of shell rather smooth. Shelf well marked. The blade arises from underside of rim of umbone and passes almost parallel to shelf. Knob prominent.

Pallets rather stout with cones very close together. Peduncle peglike and about as long as blade.

Specimens were obtained from stumps that had been under water for about a year, at Dalahican, Cavite, Luzon.

BANKIA TENUIS sp. nov. Plate 1, fig. 4.

Shell of medium size, subglobular, and thin. Anterior part fan-shaped and almost twice as wide as high. Callous rim almost straight. Ridges fine but far apart; about thirty can be counted. Anterior median rather narrow. No correlation between ridges of anterior and anterior median parts. In the latter the ridges are about twice as close as in the former, permitting irregular intercallation of the ridges of two regions. Middle median part, as usual in these animals, rather narrow and slightly sunken. Posterior median about 1.5 times as wide as anterior median part. Growth lines of middle and posterior parts well marked, widely separated, and as usual run in the opposite direction to those in anterior median. Auricle large and distinctly separated from posterior median part. Sculpture inside of the shell clear. Shelf rather low; blade springs from rim of the underside of umbone, is short, and runs twisted at an angle parallel to the shell. Knob prominent.

Pallets long and white, with cones closely approximating each other. Free margins of cones clearly pectinate or comblike; the pectination clearer in old specimens.

Collected by R. S. Filoteo, from the Cebu region.

BANKIA GLOBOSA sp. nov. Plate 1, fig. 5.

Shell small, subglobular, and thin. Callous growth along the dorsal anterior edge of anterior part runs in a straight line. Anterior part well developed. Ridges distinctly denticulate and rather coarse; over thirty can be counted. Anterior median distinctly separated from anterior. Ridges of the two regions poorly articulate with each other; those of anterior median lying closer to each other. Denticles more clearly marked in anterior median than in anterior part. Middle median narrow and marked with fine lines of growth. Posterior median about the same width as anterior median; lines of growth clearly seen and continue into the auricle. Auricle rather small; it begins at the level at which anterior part ends and extends into the ventral half of the shell.

Inside of shell clear. Shelf rather shallow. The blade springs from the rim near the umbone. It is short and straight, and flattened in a plane parallel to the edge of posterior median.

Pallets with well-fused cones. The composite nature of the pallet is more distinct from the inner side. The peduncles taper to a point. The boundary between the free and embedded parts of the peduncle is distinctly marked.

The specimens were collected from old piles at Sir J. Brooke Point, Palawan.

BANKIA RUBRA sp. nov. Plate 1, fig. 6.

Shell small, triangular, and thin. Width of anterior part exceeds its height. Callous anterior dorsal line strongly curved. Ridges evenly distributed and of medium size; about fifty were counted. Anterior median part as wide as posterior median. Ridges fine and most of them take origin at the ends of ridges of anterior part. This coördination, however, is not perfect. Dividing line between anterior and anterior median parts very sharp. Middle median a narrow groove; posterior median smooth. Auricle medium in size and curves slightly outward.

Inside of the shell the shelf is small and is best seen in the ventral region. The blade projects from under the rim of the umbone. It is a small structure and runs along the plane of the shell in such a way that the plane of the blade is almost at right angles to that of the shell.

Pallets of a loose cone-in-cone type. The cones are delicate, and their rims are beset with spines. These are of two types; namely, small toothlike spines along the inner rim of the cone (over ten of these can be counted), and two long stout spines

on the extreme margins of the cones. One of these is usually long, another much shorter. The outer margin is smooth.

The specimens of this animal were collected from living mangrove stems. This possibly accounts for the dark red color of all parts of the animal. The species is common near Puerto Galera, Mindoro.

Genus **BACTRONOPHORUS** Tapparone Canefri, 1877

Pallets large, stiltlike. From the cup of the distal end of the pallet springs a long, flat, bladelike style. This style is flat and smooth on the inner surface and lightly convex on the outer. The outer surface usually is warty.

Two species of this genus have been reported from Australia. The two described below are the first Philippine representatives.

BACTRONOPHORUS EDULIS sp. nov. Plate 2, fig. 7.

Shell comparatively large, subglobular. The anterior part is of an isosceles triangle type. Under the umbone a strongly developed callus which ends in an inward reflected knob. This knob fuses with a strongly developed shelf. Anterior part slightly wider than high. Ridges run fanlike; they are rather coarse, finely denticulate; from thirty to fifty ridges were counted in different specimens. Anterior median part narrow; less than half of middle and posterior median combined. Ridges from the anterior part continue directly into anterior median part as an obtuse triangle. Middle median a narrow strip and, as usual, darker in color; slightly elevated from surrounding region. Posterior region smooth and shows shallow lines of growth. Auricle large, runs at first at a slight angle to the posterior part, then bends sharply outward. Shelf along posterior median and auricle strongly developed. Another shelf runs along the border of the anterior and anterior median parts. Two shelves meet just under the knob, forming a deep angle at the place of junction. Some distance ventrally from this angle, from the middle of the posterior shelf, arises a thin, flat blade. Ventral knob large.

Pallets of typical *Bactronophorus* type; shorter and stouter than in either *Bactronophorus thoracites* (Gould) or *B. australis* (Wright). Blade resembles a certain type of scalpel with one straight and the other slanting edge.

The specimens were sent by Mr. J. Meñez, from New Washington, Capiz Province, Panay. I am told that in that place they are very abundant in dead logs along the shore. They

are collected, and eaten, either raw or cooked. They taste like oysters. At certain times they are pickled in vinegar and sold in nearby markets.

BACTRONOPHORUS FILOTEOI sp. nov. Plate 2, fig. 8.

Shell triangular and comparatively large and heavy. Umbone with a marked callus projecting inward. A small, thin, flat blade, at least 1 millimeter long, projects from the underside of callus. Anterior part is of an irregular rectangle type, with fine ridges; a little over one hundred twenty ridges counted; few of them eroded. Anterior median part about equal or slightly wider than posterior median part. The ridges of the anterior median are continuous with those of the anterior part; but on account of the anterior median being narrower than the anterior part, the ridges are closer together in the former than in the latter. Middle median part a narrow brown strip, slightly elevated. Posterior median markedly convex in its dorsal region, less so in the ventral. Auricle sharply marked from posterior median and runs at a slight angle to the latter.

Inside of the shell there are two shelves with a triangle between them. The triangular area in this species is shallower than that in *B. edulis*. The blade springs from the main or the posterior auricular shelf at the level where the auricle on one side and anterior part join their respective sides of the median part. The undersurface of the main shelf is filled with an irregular calcareous mass. The ventral knob as usual is prominent.

Pallets rather small and slender. Blade slightly shorter than the rest of pallet; both edges curved.

This species differs from *B. edulis* mainly by a finer ridging of the anterior part, by having anterior median and posterior median parts about equal in width, by shallower shelves, and by the size as well as the general shape of the pallets.

One specimen was sent from Cebu by R. S. Filoteo, in whose honor I have the pleasure of naming the species.

Genus **TEREDO** Linnæus, 1758

Pallets either spoon-, paddle-, or scoop-shaped. They may be cupped or not or they may even bear at their termination a calcareous knob which is usually small.

TEREDO OBTUSA sp. nov. Plate 2, fig. 9.

Shell large, heavy, and obtuse. Umbone with usual callus. Anterior part triangular; base of triangle and side about equal. Over one hundred finely denticulated ridges were counted on the anterior part. Anterior median part is slightly larger than

middle and posterior median combined. The fine ridges in this part are denticulate and appear to be closely correlated with those on the anterior part. Middle part narrow and slightly elevated. Posterior median part about half as large as anterior median; it sinks downward beginning with middle median and curves merging into auricle. Auricle obtuse, thin and transparent, of medium size and much wider than high. Inside outline of the shell deep. Shelf prominent. The blade arises at junction of the shelf and the callus knob of umbone. Blade very small. Ventral knob present.

Pallets spoon-shaped. Blade slightly excavated inside in the proximal region and convex outside. Distal regions of both sides rough. Stalks heavy and vary considerably in shape. Just before the rough area there is a periostracal band so that the pallet appears banded.

Collected from an old pile at Sir J. Brooke Point, Palawan.

TEREDO VARIEGATA sp. nov. Plate 2, fig. 10.

Shell medium in size, subglobular, and thin. Anterior part, roughly, has the shape of an equilateral triangle or that of an open fan. Height and base about equal. Ridging medium fine, with wide spaces between ridges; about forty ridges were counted. The ridges form an obtuse triangle with those of the anterior median part and at the junction they do not co-ordinate with each other. Anterior median about half of middle and posterior median combined. Ridges of anterior median fine and close together. Middle median narrow and slightly depressed. Posterior median slightly depressed and strongly marked from the auricle, which is still more depressed and of comparatively large size.

Shelf merely a ridge. The blade springs from the rim of the callus region and runs in the anterior median direction with the flat side almost at right angle to the shell. Knob of normal size.

Pallets spoon-shaped, short, and stout. The dark band of periostracum is present. In general the pallets resemble those of *T. obtusa*, but they are smaller and finer. This species differs from the preceding especially in the relative size of the parts of the median shell.

Few specimens were collected by R. S. Filoteo, in Cebu.

TEREDO PRINCESAE sp. nov. Plate 2, fig. 11.

Shell small, triangular, and thin. Anterior part triangular. Base of triangle longer than height. Ridges run in the form of a fan; coarse and distinct; about twenty were counted. An-

terior median very narrow; less than middle median. Ridges very close together and apparently coördinate with those of anterior median. Middle median prominent, slightly elevated. Posterior median about three times as wide as anterior and middle median together. Auricle very large and strongly bent outward.

The inside of the shell shows a very pronounced shelf at the junction of the auricle with the posterior median. The shelf is so prominent as to give an appearance that the auricle was placed by the side without fusion. At the junction of the callus and the shelf, springs the blade which runs parallel to the middle median part of the shell.

Pallets leaf-shaped, with a thin and broad expanded portion well cupped. No partition, stock short and very delicate. Pallets in their entirety very delicate and thin.

The specimens were collected at Puerto Princesa, Palawan, from a piece of wood that was exposed during low tide.

TEREDO BARTSCHI sp. nov. Plate 3, fig. 13.

Shell bluish white, small, thin, and brittle. It is of an isosceles triangle type with the dorsal line of articulation as its base. Anterior part and auricle large. Anterior part of an irregular quadrangle type; about as wide as high. Ridges of medium size and rather coarse; over forty were counted. Anterior median part slightly less than width of middle and posterior median combined. Ridges of anterior median meet those of anterior part at an angle slightly larger than 90°. They are continuations of each other. Middle median narrow; slightly depressed and indistinctly separated on both sides. Posterior median distinctly convex. Auricle of medium size; bent outward, forming an obtuse angle with posterior median part.

Inside smooth and bluish white. Shelf poorly developed. The blade springs from edge of callus and projects downward, running parallel to middle median part.

Pallets strong and stout; with a single cup. A poorly developed knob in the proximal edge of cup.

The tips of siphons are free and about equal in size. The calcareous tube instead of being single as in most of the tere-dos, is divided by a partition. This species and the one that follows apparently belong to the group of tere-dos represented by *Teredo arenaria*, *Teredo manni*, etc., which has been under discussion by zoölogists for generations.

The specimens were collected at Sir J. Brooke Point, Palawan, from old piles.

TEREDO DUBIA sp. nov. Plate 3, fig. 14.

Shell low, wide, large, and thin; in the adult stage it becomes very narrow, unlike that of the other teredos. In young stages the shell has the general characteristics of the shell of any other animal in this group, but even the young shell does not have a typical boundary line between the anterior and the anterior median part. In older specimens this boundary line becomes entirely eliminated. In younger specimens few ridges running from the anterior directly into the anterior median part are visible. Ridges coarse and wide apart. Middle median very narrow and marked by a seamlike elevation. Posterior median part deeply eroded and smooth. Auricle recognizable as such only in very young specimens; in adults it is almost obliterated. A strong callus just under the umbone; it is large and projects in a dorsal median direction. From the middle of this callus springs a strong blade; it is straight and peglike in young specimens, but becomes curved in various directions forming an irregular corkscrew in some of the older specimens; its general course is parallel to the shell. The rim of the inner anterior and the anterior median borders is thickened in a seamlike manner. The usual ventral knob is relatively poorly developed.

Pallets strong, paddle-shaped, with short cups in which there is a low median partition.

Calcareous tube large and heavy. The whole animal and the tube may grow to almost half a meter in length and may become of considerable thickness. It grows in hard sand. The distal end of the tube is 5 centimeters under the surface. The tube sinks straight into the ground for a considerable distance; the anterior end downward, the pallets and the siphons upward. The siphons are exposed to the surface and when seen for the first time may be mistaken for actinians or almost any other cylindrical animals. The true nature of the animal is revealed after considerable digging.

A large colony of these animals was found near Puerto Galea, Mindoro. In this colony the young and the old animals live near each other. All of them are buried in sand. This apparently is contrary to W. T. Calman's suggestion¹ that the

¹ *Nature* 119 (1927) 104.

sand-boring forms may be the fullgrown animals of the timber-boring species which are set free by the decay of the wood. This, however, does not prove that the sand-boring teredos are not related to the wood borers. The pallets of this animal in all respects, except the size and few specific characteristics, resemble those of *T. bartschi* (fig. 13) and also the pallets of an unknown species shown in fig. 12. Both of these species were taken from wood and at distant localities; the first in Brooke Point, Palawan, the second in Dalahican, Cavite, hundreds of kilometers apart. The pallets shown in fig. 12 were found in a mangrove stump. As I realized the importance of the specimen, every effort was made to dig out the entire animal; but it went so deep into the stump, going straight into the ground, that with the instruments in our possession we had to abandon the work. It is possible for such an animal to remain in the sand when the wood disintegrates.

I consider that these three species are related to *Teredo arenaria* and *T. manni*. The relationship of these animals, especially that of *T. arenaria* (sometimes called *Kuphus arenarius*), has been under discussion for generations. Until some one compares the shells and the pallets of these animals the discussion will not end. That the sand-boring teredos have very close relatives among the wood-boring forms is clear.

TEREDO HYDEI sp. nov. Plate 3, fig. 15.

Shell subglobular, large, and heavy. Sinus well developed. Umbone with the usual callus bending downward. Anterior part broad and high. Width to height ratio 3:2. It resembles an irregular quadrangle. Ridges medium fine; number, as counted, not less than one hundred fifteen. Anterior median part and middle median part together almost equal to posterior median part. Ridges of anterior median finer than those in anterior part, but they coördinate with each other. Middle median part well defined on anterior side, less so on posterior. Posterior median slightly depressed. Auricle small.

Shelf short but well defined; the blade springs from shelf at a considerable distance from umbone. Middle median distinct. Knob medium in size.

Pallets of a scoop type, wide and long, deeply concave on inside and convex on outside. Junction of peduncle with blade marked by an elevation running on the concave side of blade. The pallets have a smooth surface, though they may show concentric lines of growth. They are cream white at all stages.

These animals were found in large numbers in mangrove stumps on the shore at Dalahican, Cavite. I have the pleasure of naming this species in honor of Dean Edward R. Hyde.

TEREDO APENDICULATA sp. nov. Plate 3, fig. 16.

Shell small, thin, and triangular; anterior part slightly wider than high. Ridges far apart; thirty were counted. Anterior median part about half as wide as posterior median and middle median combined. The ridges are roughly denticulate and do not coördinate entirely with those of the anterior part. Middle median part narrow. Auricle large and reflected outward.

The shelf may be well developed. The blade springs from shelf at some distance from umbone and runs parallel to shell. Knob of medium size.

Pallets of scoop type. Peduncles very short or lacking. The insertion lines are marked by two grooves on the proximal end of the pallets. The pallets are smooth and cream white in living and in preserved specimens.

This species resembles *T. hydei* but is smaller, possesses rougher sculpture, has much narrower anterior median, and has pallets without insertion stalks. The last phenomenon has never been found in any other teredo reported. I am satisfied that they were not broken.

The specimens were collected from pieces of wood exposed at low tide in Puerto Princesa, Palawan.

TEREDO PARKSI Bartsch. Plate 3, fig. 17.

Teredo parksi BARTSCH, Proc. Biol. Soc. Wash. 34 (1921) 25-32.

This species is apparently common in the Philippines. The specimens illustrated in fig. 17 were collected from floating wood in Batag Bay, Samar. The animals are very small.

Besides this collection we have in the departmental collection two more vials with the animals of this species. The locality is not given, but presumably they were collected somewhere in the Philippines.

The animals of this species have been described by Bartsch and by Miller.² Miller reports the species from Cavite.

THE MORE IMPORTANT LITERATURE ON SHIPWORMS

BARTSCH, P. A new classification of the shipworms and descriptions of some wood-boring mollusks. Proc. Biol. Soc. Wash. 34 (1921) 25-32.

BARTSCH, P. A monograph of the American shipworms. Bull. U. S. Nat. Mus. 122 (1922) 1-51.

² Univ. Calif. Pub. Zool. 26 (1924) 146.

- BARTSCH, P. The shipworms of the Philippine Islands. Bull. U. S. Nat. Mus. 100 2° (1927) 533-554.
- MARTENS, E. VON. Süß- und brackwasser-Mollusken des indischen Archipels. Zoöl. Erg. Niederl. Ost-Indien. Max Müller 14 (1897) 1-331.
- MILLER, R. C. Variations in the shell of *Teredo navalis* in San Francisco Bay. Univ. Calif. Pub. Zoöl. 22 (1922) 293-328.
- MILLER, R. C. Wood-boring mollusks from the Hawaiian, Samoan, and Philippine Islands. Univ. Calif. Pub. Zoöl. 26 (1924) 145-151.
- WRIGHT, E. P. Contributions to a natural history of the Teredidae. Trans. Linn. Soc. 25 (1866) 561-568.

ILLUSTRATIONS

[Figs. 1, 4, 8, 10, and 15 were taken by Prof. José I. del Rosario; figs. 2, 3, 5, 6, 7, 9, 11, 12, 13, 14, 16, and 17 by Mr. Juan Fontañoza. The figures vary in scale. Where the scale is not given one exact measurement is given in the explanation; any other measurements can be easily computed. Where the scale is in the figure it is always in millimeters.]

PLATE 1

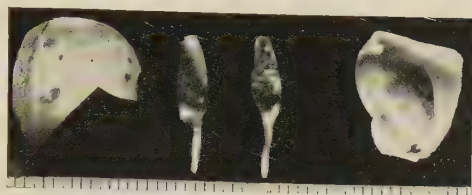
- FIG. 1. *Bankia triangularis* sp. nov.; outside and inside views of the shells and the pallets.
2. *Bankia oryzaformis* sp. nov.; outside and inside views of the shells and the pallets. The pallet in the center is 10 millimeters. Other sizes in the same ratio.
3. *Bankia quadrangularis* sp. nov.; outside and inside views of the shells and the pallets. Actual length of the pallet on the left is 20 millimeters. Other sizes in the same ratio.
4. *Bankia tenuis* sp. nov.; outside and inside views of the shells and the pallets.
5. *Bankia globosa* sp. nov.; outside and inside views of the shells and the pallets. The actual length of the pallet on the right is 8 millimeters. Other sizes in the same ratio.
6. *Bankia rubra* sp. nov.; several views of the shells and the pallets. The actual length of the pallet on the extreme left is 9 millimeters. Other sizes in the same ratio.

PLATE 2

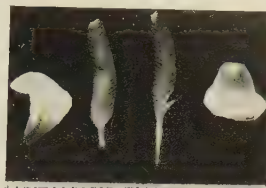
- FIG. 7. *Bactronophorus edulis* sp. nov.; shells and pallets. Note the narrow anterior median part. The actual length of the pallet on the left is 16 millimeters.
8. *Bactronophorus floteoi* sp. nov.; shells and pallets. Note the wide anterior median part and the position of the blade.
9. *Teredo obtusa* sp. nov.; various views of the shells and the pallets. Note the wide anterior median part and very close ridging on the anterior part. The actual length of the pallet on the left is 14.5 millimeters. Other sizes in the same ratio.
10. *Teredo variegata* sp. nov.; shells and pallets. Note the narrow anterior median part and the coarse ridging on the anterior part.
11. *Teredo princessæ* sp. nov.; shells and pallets. The actual length of the longest pallet on the right is 3.3 millimeters. The other sizes in the same ratio.
12. *Teredo* sp.; pallets. Compare their shape with the two following species. The actual length of the longer is 16 millimeters. Other sizes in the same ratio.

PLATE 3

- FIG. 13. *Teredo bartschi* sp. nov.; shells and pallets. The piece of tube shows double perforation for the exit of siphons. This type of tube is characteristic of this group of *Teredidæ*. Actual length of the first pallet on the left side is 8.25 millimeters. Other sizes in the same ratio.
14. *Teredo dubia* sp. nov.; group of shells and pallets. Paired shells and pallets shown in the center are of the smallest specimen found. Note the erosion and a peculiar ridging of the shell.
15. *Teredo hydei* sp. nov.; shells and pallets. Note the width of the anterior median and rather fine ridges on the anterior and the anterior median. Note also the stalks of the pallets.
16. *Teredo apendiculata* sp. nov.; shells and pallets. Note the narrow anterior median part and rather coarse ridging of the anterior part. Note also the absence of stalks. Actual length of the pallet on the right is 8.5 millimeters. Other sizes in the same ratio.
17. *Teredo parksi* sp. nov.; shells and pallets. Actual length of the pallet on the left is 6.6 millimeters. Other sizes in the same ratio.



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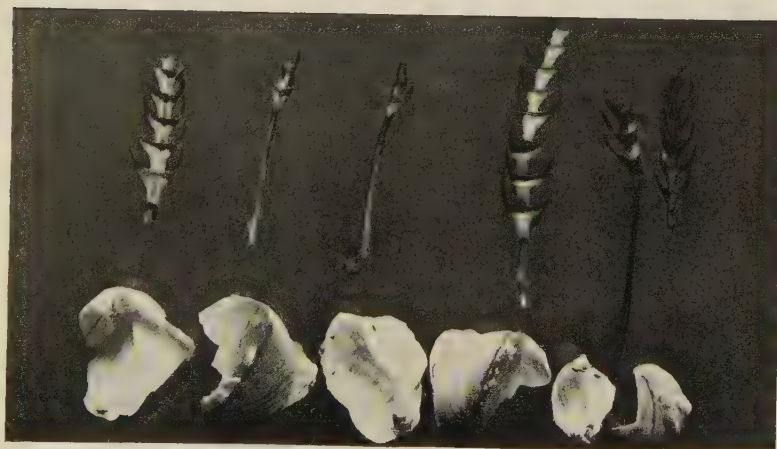
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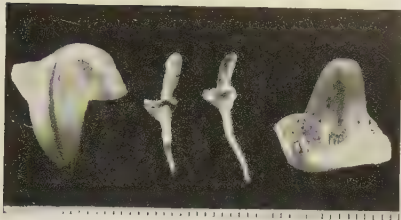
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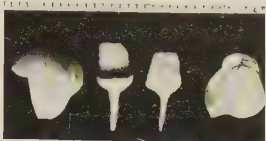
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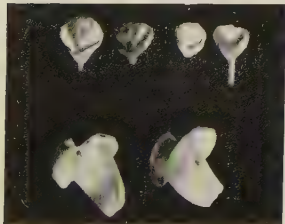
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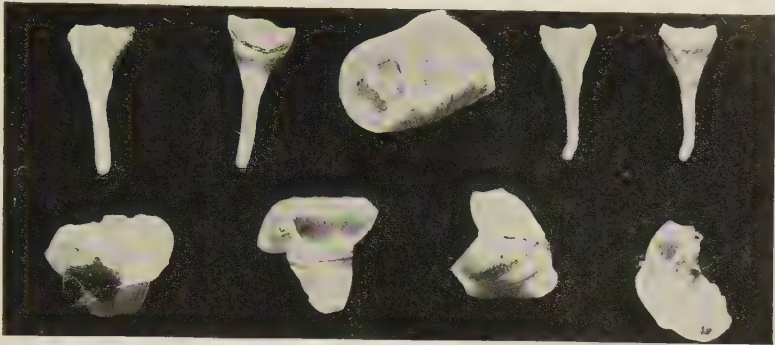
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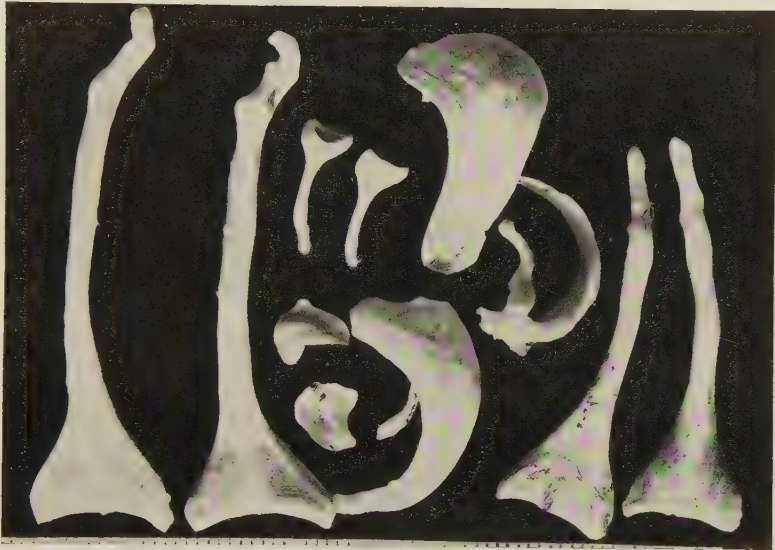
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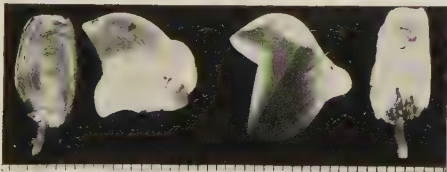
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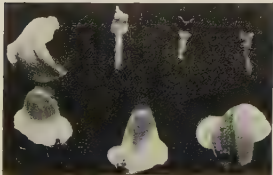
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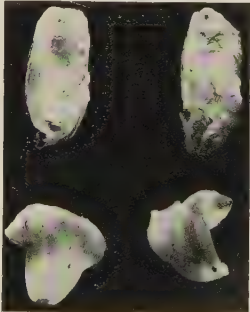
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THE MORPHOLOGY OF A HOLOTHURIAN, STICHOPUS CHLORONOTUS BRANDT

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ELEVEN PLATES

INTRODUCTION

Stichopus chloronotus was originally described by Brandt in 1835 from specimens collected in Guahan (Guam). He placed it in the subgenus *Perideris*. Selenka (1867) in his study of similar animals collected from Zanzibar, Africa, and the Sandwich Islands, as well as Ludwig (1882), who later studied those collected from the Indian Ocean; Mauritius, Africa; and Macassar, Celebes, agreed with Brandt in their identification. Haacke (1880), however, described similar specimens collected from Mauritius under a new name, *S. cylindricus*. Later Théel (1886), Kent (1893), Fisher (1907), and Clark (1921) concurred with Brandt, Selenka, and Ludwig in their description of similar animals collected from the Friendly Islands, Australia, Hawaii, and Torres Strait.

Clark (1921) states that the geographical range of *S. chloronotus* is from Mozambique, Africa, to the Hawaiian Islands. It seems that *S. chloronotus* is confined to the Indo-Pacific region and is commonest in the East Indies and neighboring places. As far as available literature shows, Théel is the only worker on this group of animals who mentions the presence of *S. chloronotus* in the Philippines. Théel's statement is vague, since he gives neither the exact location nor a further description of the species.

Selenka (1867) appears to have been the first to work on the anatomy of *Stichopus chloronotus*; but his work is far from being complete. Semper (1868) worked on the anatomy of different forms of Philippine holothurians but did not work on *S. chloronotus*. However, he studied *S. variegatus*, which though possessing many differences in some respects is very similar to *S. chloronotus*. Théel (1886) confined himself exclusively to the spicules of *S. chloronotus*. It seems that *Sticho-*

pus chloronotus has never been adequately studied. The purpose of this paper is to describe the gross and the microscopic anatomy of this animal.

HABITAT

In the Philippines *S. chloronotus* has been collected from Puerto Galera, Mindoro, and from around the Hundred Islands, Lingayen Gulf, Pangasinan Province, Luzon.

In the vicinity of Puerto Galera, Mindoro, *S. chloronotus* is most abundant along the sandy shores of Puerto Galera Bay, especially in the Northwest Channel along the Paniquian coast of Medio or San Antonio Island. In Lingayen Gulf it appears to be less common. In both places it is a littoral form, living in water less than a fathom deep. In some places at Puerto Galera the animals are so common that they can be collected by the thousand. Occasionally individuals are found in grassy regions; never in muddy places. Usually they do not take shelter under rocks and corals and are exposed during low tide.

EXTERNAL CHARACTERISTICS

The animal is elongate, quadrangular, and somewhat tapering at both ends (Plate 1, fig. 1). Its mouth is slightly on the ventral side of the anterior end (Plate 1, figs. 1 and 2), and the anus is at the posterior. The adult ranges from 200 to 400 millimeters in length. In the middle of the body the width may reach 75 millimeters and the height 50. The largest specimens reported by Kent (1893) and by Clark (1921) do not exceed 300 millimeters.

Authorities differ in the description of the color of this animal. Lampert (1885) described it as olive green; Théel (1886), olive brown; Kent (1893), clear bottle green with the tips of the papillæ orange or scarlet; Fisher (1907), olive brown; Mitsukuri (1912), deep black with the tips of the papillæ bluish and ocher yellow; Clark (1921), deep green or almost black with the tips of the papillæ brown orange. The Puerto Galera and Lingayen Gulf specimens are deep green with lighter irregular bands under sunlight with the tips of the papillæ slightly lighter or orange in color. One specimen, however, was found with the body distinctly lighter in color than usual and with the papillæ banded with deep orange. The orange tips of the papillæ can be extended and retracted.

The interradial spaces are naked and plain. The dorsolateral and ventrolateral radii are provided with papillæ alternately arranged (Plate 1, fig. 1). This arrangement is distinct

in the former. The ventral surface shows that it is formed by three of the five radii and is provided with three rows of pedicels or tube feet. The middle row of tube feet is twice as wide as the lateral ones (Plate 1, fig. 2). The difference in width of these three rows of tube feet is due to the fact that the middle row takes up an entire radius while each of the lateral ones occupies only half of the radius, the other half is taken by the ventrolateral papillæ. The distinct alternate arrangement of the dorsolateral papillæ is due to the absence of the tube feet in this region, so that the entire radius is left to the papillæ.

Stichopus chloronotus has twenty tentacles. A few specimens are recorded with eighteen or nineteen tentacles only. The stalks of the tentacles are cylindrical, resembling an Indian club. They are usually stouter at the base and are 15 to 25 millimeters in length and 4 to 7 millimeters in diameter at the widest part. The distal end is disk-shaped or more or less like a powder puff (Plate 1, fig. 2). Close examination of the disk shows four main branches, each one divided and subdivided into many smaller branches, thus giving the appearance of a powder puff. The bases of the tentacular stalks are arranged in a circle and are united with each other at the margin of the peristome (Plate 1, fig. 2). The tentacle is provided with several types of spicules which are described under the skeletal system.

THE SKELETAL SYSTEM

The skeletal system in different parts of the body consists of variously shaped spicules, ranging from 37 to 796 microns. Scattered within the body wall are the simple tables with four short pillars (Plate 2, figs. 22-29) and C-shaped spicules (Plate 2, figs. 30, 31) about 37 microns in their longest axis. The simple tables are embedded in the pigment layer of the body wall and are superficial in position (Plate 9). The C-shaped spicules are scattered in the connective-tissue layer (Plate 9).

In the papillæ are found the same types of spicules as in the body wall with the addition of another type in the form of rods (Plate 3, fig. 19). These rods, which are either curved or branched, and average 370 microns in length, strengthen the distal portion of the papillæ.

The tentacles are provided with many forms of spicules. The stems of the disk-shaped part of the tentacles have small quadrangular rods (Plate 2, figs. 3, 4). The distal part of the main stalk of the tentacles contains large quadrangular rods

slightly racemose at both ends. These rods are either curved or straight and range from 647 to 796 microns in length (Plate 2, figs. 1, 2). The general arrangement of these rods is at right angles to the longitudinal axis of the stalk. The majority of these rods are curved and follow the curvature of the cylindrical stem. Proximally there are very numerous small rosettes; some are simple and incomplete, while others are complex and appear like small sieve plates (Plate 2, figs. 10-16). These are scattered all over the wall of the main stem of the tentacle. In the proximal half of the stalk the supporting rods are entirely wanting and the rosette forms are fewer. In this part the spicules in the form of large tables predominate (Plate 2, figs. 5-9). The majority of these tables have six pillars, although some smaller ones have only four (Plate 2, fig. 5). They range from 56 to 148 microns in height. Their bases are perforated like those of regular sieve plates (Plate 2, fig. 6). Their pillars are connected with each other by crossbars and are spinous or racemose at their tips. At the proximal end, next to the rim of the mouth, are numerous C-shaped spicules, which are the typical body-wall spicules.

At the very tip of the tube feet are large supporting sieve plates that lie close together, and the group appears like a single large sieve plate. Those in the central portion have no definite shape; some are circular, others are irregular (Plate 3, fig. 2), while those at the margin or rim of the tip are uniform in size and shape (Plate 3, fig. 1). They are usually characterized by two large holes at the center surrounded by several smaller ones. At the sides, near the tip, there are many spinous supporting rods. Some of these rods are similar to those of the papillæ, but the majority are branched at the middle part, usually on one side (Plate 3, figs. 16-19). These side branches at the middle vary from simple spinous processes to complex sieve-plated ramifications. The latter are usually accompanied by slight ramifications on the opposite side (Plate 3, figs. 16, 17). They are more or less uniform in size. The average length of these rods is 333 microns. They are arranged parallel to each other at right angles to the longitudinal axis of the pedicel. They are usually found beneath the epidermis in the connective-tissue layer. Near the surface there are many small simple tables which are scattered all over the proximal part of the pedicel. Some of these tables have short pillars (Plate 2, fig. 25). Aside from all these spicules in the tube feet there are C-shaped ones.

Even the internal organs are provided with spicules and calcareous deposits. The calcareous ring, the stone canal, and the madreporite are filled with calcareous material usually in the form of a reticular network. In the walls of the water-ring vessel, in the Polian vesicles, and in the ampullæ of the tentacles there are numerous rodlike spicules arranged parallel to each other (Plate 3, figs. 6-8). The suspensors of the pharynx are provided with similar spicules. The gonads have ><-shaped spicules and few rodlike ones (Plate 3, figs. 3-5). The trunks of the respiratory trees possess complex rosettelike spicules, which appear like small sieve plates and range from 110 to 190 microns in diameter (Plate 4, figs. 5, 6). Those at the distal part are mixtures of simple and complex forms with various sizes from the minimum to the maximum, while those at the proximal are uniformly of the maximum size. The wall of the alimentary canal is also provided with numerous rodlike, ><-shaped, and some X-shaped spicules scattered from the pharynx to the cloacal region (Plate 3, figs. 10, 11). When intestinal appendages are present they also have some rod-shaped spicules in their walls, especially those that are just beginning to protrude from the wall of the canal.

The calcareous ring is made of well-developed calcareous plates fused to each other and surrounding the pharynx (Plate 3, figs. 20, 21; Plate 5, fig. 1; Plate 6). There are twenty plates fused together to form a ring. Five of these plates, called radial pieces, lie at the anterior ends of the five pairs of radial longitudinal muscles at the place of their attachment (Plate 3, fig. 20; Plate 7, fig. 3). The other fifteen are called interrarial pieces (Plate 3, fig. 21). The two dorsal radial pieces are provided with conspicuous, bifurcate, posterior prolongations and are better developed than those of the ventral. The interrarial pieces are all symmetrical and smaller than the radial. They are slightly concave behind and have a single median point in front (Plate 3, fig. 21). On each side of this median point the plate is hollowed for the reception of a tentacular ampulla and for the attachment of the basal part of the tentacular canal. According to Clark (1907) these plates arise from calcareous bars, which branch more or less irregularly; the branches anastomose and fuse until solid plates are formed. The plates are somewhat triangular in form. It seems as if there is an agreement between the number of the tentacles and the total number of plates in the calcareous ring. This is shown by the fact that the tentacular ampullæ are of the same number as the radial

and the interradial plates. The anterior part of each of the radial plates bifurcates, producing an indented part for the passage of the radial nerve cord from the circumoral nerve ring (Plate 3, fig. 20; Plate 5, fig. 1). Microscopically these plates are not homogeneous but are made up of a reticular network of calcareous filaments, or bodies, similar to that of the stone canal (Plate 8, figs. 1-6). This reticular network of calcareous bodies is intermixed and inclosed by fibrous connective tissue.

THE BODY WALL

The body wall of *Stichopus chloronotus* is fairly thick but not firm, and the thickness is almost uniform throughout (Plate 6). As in other holothurians it consists of five parts (Plate 9); namely, a cutis and a layer of epithelial cells which make up the epidermis; a layer of connective tissue in which are located the calcareous deposits, pigments, and some spheruliferous corpuscles; a layer of circular muscles; two radial bands of longitudinal muscles for each ambulacrum; and an inner epithelium which lines the body cavity.

Epidermis.—The outermost part of the epidermis, or cutis, is a thin homogeneous layer secreted by the epithelial cells. The epidermal epithelium is composed for the most part of two kinds of cells, the supporting cells and the sensory cells. The supporting cells are the most abundant and they make the greatest bulk of the epidermis. They are usually columnar cells tapering toward the connective-tissue layer (Plate 8, figs. 11, 12). In addition to these there are a few clear glandular cells in the region of the tentacles. It is believed that this third kind of cell is similar to the gland cells identified by Clark (1907) in the epithelial cells of *Molpadidæ*. The epidermal layer in the distal part of the branches of the tentacles is much thicker than that of the side. It is about 40 microns in thickness as compared with that of the side which is only about 18 microns thick (Plate 7, fig. 2). This is due to the greater number of the supporting and sensory cells. Most of the sensory cells are spindle in form (Plate 8, figs. 11, 12). Each of the minute branches of the tentacle is really an association of these sensory cells with some supporting cells similar to the touch papillæ in *Molpadidæ* as found by Clark (1907).

Connective-tissue layer.—The connective-tissue layer forms the greater part of the body wall (Plate 9). Unlike that of

other holothurians, the thickness of this layer is practically uniform throughout. This is the layer in which most of the calcareous deposits are lodged. A small piece of this layer when examined fresh under the microscope reveals many connective-tissue fibers with some spheruliferous and oval corpuscles (Plate 8, figs. 9, 10, 15). The oval corpuscles vary in size from 30 to 37 microns, while the spheruliferous are more or less uniform, about 23 microns in diameter. The connective-tissue fibers, like those of other holothurians, are prolongations of cells embedded in a homogeneous matrix. They are very long, and the ends are split into many fibrils. It is possible that many of the fibers may have no cellular connections because of the abundance and the length of fibers compared with the small number of cells. Histologically the superficial fibers are loose and spongy, filled with spheruliferous and oval corpuscles, some pigment cells, and few spheruliferous wandering cells. The inner or deeper layer is closely filled with the fibers running almost parallel to each other. The nuclei of a few cells are seen among those fibers together with some corpuscles scattered in a limited number. In the tentacle in this particular layer are the supporting rods. The perpendicularly arranged muscular tubules are also found in this layer. These, however, are limited to the dorsal region.

Muscular layer.—The circular-muscle layer runs almost completely around the body cavity, except in the ambulacral regions just external to the lining epithelium (Plates 6 and 9). It is uniform in thickness throughout, except at the approach of the cloacal opening where it becomes better developed and forms a sphincter. This sphincter partially closes and opens the anus during respiration, allowing the water to enter and leave the respiratory trees.

The radial longitudinal muscles are arranged in pairs, one on each side of the radial water vessels. Anteriorly the paired bands of muscles are very close to each other, where they form a single tube attached to the radial plate of the calcareous ring (Plate 7, fig. 3). Posteriorly the muscles of each radial pair become reduced. They taper gradually until they terminate at the sphincter, aiding the latter in the opening and the closing of the anus during the respiration. The median edges of the paired, radial, longitudinal muscles are attached to the lateral wall of the radial water canal, while the outer edges are free

(Plate 7, fig. 1; Plate 9). The entire ventral side of this muscle layer is attached by connective-tissue strands to the circular-muscle layer (Plate 10, fig. 4).

Lining epithelium.—The inner epithelium, commonly called the coelomic epithelium, is the innermost layer of the body wall. It is composed of flat polygonal cells. It lines the circular and radial longitudinal muscles as well as the radial water canal and the ampullæ of the tube feet (Plate 7, fig. 1; Plate 9). Its thickness is more or less uniform, about 18 microns, except at the ampullæ of the tube feet where it is composed of a single layer of flattened cells about 5 microns thick.

THE BODY CAVITY

The body cavity of *S. chloronotus*, like that of other holothurians, is spacious, extending from the oral disk to the posterior tip of the body without any marked separation into parts. Longitudinally the cavity is incompletely traversed by three mesenteries, which suspend the three coils of the alimentary canal. These are the dorsomedian mesentery, suspending the stomach and the anterior portion of the small intestine; the lateral mesentery, supporting the rest of the small intestine; and the ventral mesentery, supporting the large intestine, or the posterior part of the alimentary canal. Posteriorly it is traversed by strands of connective tissue with muscle fibers supporting the cloaca (Plate 6). The body cavity is lined with a thin, flattened epithelium, which was described in connection with the body walls. The fluid content of the body cavity is largely water containing albuminous material with numerous amoebocytes (Cuénot, 1891), or wandering cells, (Durham, 1892) and some corpuscles (Plate 8, figs. 15, 16). Some of the corpuscles are similar to those found in the connective-tissue layer of the body wall. Since the body cavity is in constant communication with the water-vascular system through the madreporic body and the stone canal, it is thought that the fluid of the body cavity may be identical with that of the water-vascular system.

THE WATER-VASCULAR SYSTEM

The water-vascular system is composed of the circular canal, the five radial canals, the tentacular and ambulacral canals with their ampullæ, the stone canal, and the madreporic body.

The water-circular canal lies posterior to the calcareous ring around the pharynx and gives off five main radial vessels, which run to its inner surface (Plate 5, fig. 1; Plate 6). Unlike other holothurians, in this species the lumen of the circular

canal and the main radial vessels are large (about 5 millimeters in diameter), and their walls are thin. Both the circular and the main radial canals are attached to the outer wall of the pharynx and are supported by many connective-tissue strands with muscle fibers (Plate 6). The wall of the circular canal consists of four layers, as follows: An outer epithelial layer of cells which is continuous with the lining epithelium of the body cavity; a layer of connective tissue consisting chiefly of fibers containing a few wandering cells and rodlike spicules; a circular-muscle layer; and an internal epithelium of ciliated cells (Plate 10, fig. 3). Close to the posterior part of the circular canal and partly related to it is the pharyngeal blood ring (Plate 5, figs. 1, 2; Plate 6).

The five main radial vessels run anteriorly to the calcareous ring. They are largest at the place where they leave the circular canal, but rapidly decrease in size by giving off four branches from which the tentacular canals are formed. The two pairs of branches are given off at different levels, and each branch supplies a tentacle as can be seen in Plate 5, fig. 1. Each of the radial canals runs forward under the median indented part of the radial piece of the calcareous ring (Plate 5, fig. 1; Plate 7, fig. 3), then it bends outward and backward to the body wall along the inner side of the hyponeural canal extending posteriorly to the tip of the body where it terminates in a rudimentary ambulacral appendage. Histologically the wall of the radial canal differs from the circular canal chiefly in the absence of a circular-muscle layer. The tentacular canals, which are really the side branches of the radial canals, differ histologically from the ring canal in the presence of valves and a thin layer of longitudinal muscles. The same thing is true of the ampullæ of the tentacles, but their body layers are much thinner than those of the tentacular canal and they have no valves. In some of the tentacular ampullæ many dead wandering cells accumulate, which usually make the tips brownish black. These tentacular ampullæ are backward extensions of the tentacular canals outside of the calcareous ring to which they are closely attached (Plate 5, fig. 1; Plate 6). They are prolonged considerably over the posterior margin of the circular canal and hang freely in the body cavity. Each of the five radial vessels gives off side branches known as ambulacral canals. The openings of these canals from the radial vessel are provided with tiny valves. In the median, ventral, radial vessel the ambulacral canals lead to the tube feet and are known

as pedal canals (Plate 9). The medial ambulacral canals of the two ventrolateral radial vessels lead to the pedal canals, but the lateral ones lead to the papillæ and are known as appendicular canals. The ambulacral canals of the two dorsolateral vessels lead to the appendicular canals. Each pedal and appendicular canal is provided with a little ampulla which extends into the body cavity (Plate 9). Histologically the pedal and the appendicular canals are similar. Each has a connective-tissue layer, a longitudinal-muscle layer, and a lining epithelium.

Aside from the five radial vessels, which arise from the circular canal, there are four interradiar tubes in the form of a dorsal stone canal and three Polian vesicles (Plate 5, fig. 1; Plate 6). The stone canal is an unbranched tube arising from the circular canal close to the pharyngeal blood ring in the median dorsal interradius. It lies between the two layers of the dorsal mesentery. It appears as an irregular twisted tube, which runs forward and upward and terminates within the body cavity in a whitish heart-shaped madreporite body (Plate 5, fig. 1; Plate 6). This madreporite is slightly concave on one side and convex on the other with a slightly pointed apex. The entire outer region is porous and covered with ciliated epithelial cells. Internally it is divided into chambers (Plate 11). The central cavity is directly continuous with the lumen of the stone canal and opens into the body cavity through the numerous pores. The madreporite is composed chiefly of closely interlocked, irregularly branching, calcareous bodies. The stone canal consists of connective tissue intermixed with calcareous bodies and covered externally with flat epithelium of the body cavity. Internally it is lined with conspicuous, columnar, ciliated cells (Plate 10, fig. 5; Plate 11). These cells are low and cuboidal on one side, but on the opposite side they become much higher or columnar and bear prominent cilia as long as, or longer than, the height of the cells themselves (Plate 10, fig. 5).

The Polian vesicles are usually three in number, although a few abnormal cases with a large number are recorded. Usually two of the three vesicles leave the circular canal at the left ventral interradius and one at the right ventral interradius (Plate 5, fig. 1; Plate 6). In some cases this condition may be reversed, two at the right and one at the left. One abnormality recorded is a large branched Polian vesicle at the left ventral interradius together with four rudimentary ones, three of which are at the right ventral interradius. The mi-

crossoscopic structure is similar to that of the circular canal, but the circular-muscle layer is thicker.

The fluid contained in the water-vascular system, as previously noted, appears to be the same as that of the body cavity because of the close relation existing between the two through the madreporite. In the water-vascular fluid, however, there is a large number of corpuscles and wandering cells (amœbocytes).

THE ALIMENTARY CANAL

The mouth is a circular opening at the center of the oral disk, situated at the anteroventral end of the body (Plate 1, fig. 2). It has neither armature, teeth, nor papillæ and is placed in the midst of the tentacular circlet. It is connected with the stomach by a straight, short, funnel-shaped tube which is considered as the pharynx by Clark (1907) and others. Some workers, for example Sedgwick (1909), consider it the œsophabus. The anterior portion of the pharynx is somewhat larger than the posterior part and resembles an attenuated funnel. The pharynx may be completely closed anteriorly through the contraction of the well-developed sphincter muscles. Arising from its outer wall are numerous tiny connective-tissue strands with muscle fibers. These strands are impregnated with many rod-shaped spicules, which range from 37 to 92 microns in length (Plate 3, figs. 6-8). Some of these strands of connective-tissue fibers, called suspensors (Gerould, 1896), attach the pharynx to the inner surface of the calcareous ring as well as to the circular and main radial canals. Posterior to the pharynx is the stomach, which is not distinctly marked off from the intestine (Plate 6). Next to the stomach is the intestine. The first half of the intestine corresponds to the vertebrate small intestine and extends posteriorly and then turns forward and extends to almost near the anterior end of the body cavity (Plate 6). Here it bends backward ventrally to form the large intestine. It extends posteriorly to the cloaca, which opens through the anus at the hind end (Plate 6). The hinder part of the large intestine is rather abruptly enlarged where it forms the cloaca and receives the respiratory trees (Plate 6). The alimentary canal is attached to the body wall along its whole length by mesenteries, which according to some authors are derived mainly from the dorsal mesentery of the larva. This mesentery consists of a very thin sheet of connective tissue containing isolated muscle

fibers running in various directions and many spheruliferous corpuscles. The pharynx, the stomach, and the first part of the small intestine are attached to the body wall by the dorso-median mesentery to the dorsal interradius. At its first bend the mesentery passes across the left dorsolateral radius to the left lateral interradius where it supports the part of the small intestine running forward. At the second bend the mesentery passes across the intervening radii and interradii into the right ventral interradius where it supports the large intestine. The arrangement of mesenteries in general is similar to that described by Selenka (1867). Both the lateral and the ventral mesenteries are perforated by many holes, which allow a continuous flow of the body fluid.

Histologically the wall of the alimentary canal is practically the same throughout. It consists of five layers, as follows: An outer layer, or epithelium, which is continuous with the lining epithelium of the body cavity, corresponding to the visceral peritoneum of vertebrates; a thin outer layer of connective tissue; a muscular layer composed of circular- and longitudinal-muscle fibers; a thick inner layer of connective tissue; and a lining epithelium corresponding to the mucosa of vertebrates (Plate 10, figs. 2, 6).

The relative thickness of these layers, however, differs in various parts of the alimentary canal. The muscle layer of the pharynx at the posterior part contains longitudinal fibers within the circular series. The inner, thicker, connective-tissue layer is impregnated with many rod-shaped spicules, which range from 56 to 94 microns in length. The epithelial lining of the pharynx is thrown into longitudinal folds and is covered by a delicate cuticle.

In the wall of the stomach both the circular- and the longitudinal-muscle layers are well developed. The inner connective-tissue layer is vacuolated and is provided with X-shaped spicules 50 to 74 microns in height (Plate 3, figs. 10-15). The lining epithelium consists of columnar epithelial and gland cells. This layer is thrown into prominent longitudinal folds at the lateral and dorsal side of the stomach. These folds are absent on the ventral side.

In the small as well as in the large intestine the longitudinal-muscle fibers are poorly developed. A few bundles of these fibers are entangled within the outer reticular part of the inner connective-tissue layer (Plate 10, fig. 2). The circular-muscle layer, however, is well developed. The inner connective-tissue

layer of the small and the large intestine is thick and is divisible into two regions; namely, an outer, vacuolated, reticular layer with coarse fibers and in inner, homogeneous, fibrous layer with finer fibers (Plate 10, fig. 2). This connective-tissue layer of the intestine is provided with X-shaped and rod-shaped spicules. The lining epithelium is composed of columnar cells (Plate 10, figs. 2, 6). It differs from that of the anterior portion of the canal in having no gland cells. It is also thrown into longitudinal folds, which are not as prominent as those in the stomach. The folds are more or less uniform in thickness all around, so that when the intestine is full of food material, the wall is usually distended and the contents are visible from all sides of the canal.

Histologically the cloaca is similar to the other regions of the canal (Plate 10, fig. 6). The muscular layer is like that of the stomach. The inner connective-tissue layer is very thick and is composed of the same parts as that of the intestine. It possesses a mixture of rod-shaped and $><$ -shaped spicules. The epithelial lining is similar to that of the intestine. Numerous strands of connective tissue with muscle fibers that are outgrowths of the wall of the cloaca attach the cloaca to the body wall (Plate 10, fig. 6). These strands differ from the suspensors of the pharynx by the absence of spicules. Wandering cells of various forms are abundant in the internal epithelial layer throughout the alimentary canal.

Stichopus chloronotus, unlike most of the species of holothurians, has no Cuvierian organ.

THE RESPIRATORY TREES

The respiratory trees, sometimes called branchial or gill trees, consist of two main branches, the left and the right. They arise from a short common trunk on the anterodorsal part of the cloaca (Plate 6). Each main branch consists of a single main tube, which bears numerous conspicuous lateral outgrowths and extends forward close to the calcareous ring. In a few cases the left branch is longer than the right, although normally the two are practically of equal length. The greatest portion of the left branch is in close relation with the blood plexus of the dorsal intestinal vessel (see below). The right one is usually free in the body cavity, thus differing from other holothurians where it is closely attached to the body wall. The bulbular ends of the lateral outgrowth when examined fresh under the microscope can be seen to contract and relax.

This contraction and relaxation is associated with the incoming and outgoing of water through the anus during the process of respiration and is possibly caused by the alternate contractions of the circular- and longitudinal-muscle layers of the system. The water that is taken in through the anus is forced into the finest branches, which end in small enlargements, or bulbs. From these the water diffuses through the walls into the body cavity.

The respiratory tree is an outgrowth of the intestine and histologically is similar to it (Plate 10, fig. 1). The outermost layer is a thin epithelium, composed of flat and irregular polygonal cells outside a thin layer of connective tissue. Below the latter is the muscular layer, composed of circular and longitudinal muscle. Following the muscular layer is a thick connective tissue, which is vacuolated and impregnated with complex rosette-shaped spicules ranging from 110 to 190 microns in width (Plate 4, figs. 5, 6). These spicules are found only in the main trunks of the left and the right respiratory trees and are practically absent in the lateral branches. The last layer is the lining epithelium, composed of cuboidal epithelial cells. In the lumen, in some sections, are numerous wandering cells. There are no openings by which the cavity of the bulbular ends or ampullæ of the respiratory trees could communicate directly with the body cavity.

THE HÆMAL SYSTEM

The principal parts of the hæmal, or blood, system of *S. chloronotus* are the pharyngeal blood ring, the dorsal and ventral intestinal vessels, the radial perihæmal canals, and the lacunar spaces. The pharyngeal blood ring, which forms the center of the system, lies immediately behind the water-ring canal (Plate 5, fig. 1; Plate 6). Histologically it does not have the appearance of a blood ring as its lumen is divided into many chambers (Plate 5, fig. 2). Its wall is made up of connective tissue with few rodlike spicules and spheruliferous wandering cells (Plate 4). Within the chamber, however, there are groups of many blood corpuscles and few wandering cells. The blood corpuscles are practically uniform in size and shape (Plate 8, figs. 17, 18). They are circular and measure about 2 to 3 microns. Some of the chambers communicate with the water-ring canal and others with the peripharyngeal sinus. This affords a chance for the wandering cells to get into the chambers of the pharyngeal blood ring together with the blood corpuscles.

The dorsal intestinal vessel lies at the line of union of the dorsal mesentery and the alimentary canal, while the ventral vessel lies on the opposite side. The two vessels are quite distinct from the canal and are connected to the lacunar spaces of the latter only by numerous short cross vessels forming a plexus called "rete mirabile" by Sedgwick (1909). The rete mirabile of the dorsal intestinal vessels in the region of the stomach and the first part of the small intestine is a typical plexus along the vessel giving off uniform parallel short cross vessels to the lacunar spaces of the alimentary canal (Plate 6). In the region of the last part of the small intestine the dorsal vessel gives off numerous long fine branches forming a great plexus which almost entirely inwraps the left respiratory tree (Plate 6). There is a close relationship between the plexus and the branches of the respiratory tree, the latter being inclosed or surrounded by the former (Plate 4, fig. 3). Beyond this the main vessel runs posteriorly and gives off several shorter and stouter cross vessels to the intestine. Then it lies very close to the canal in the region of the large intestine and disappears into lacunar spaces.

The ventral hæmal vessel passes from the pharyngeal blood ring to the posterior part of the alimentary canal without forming a plexus with the respiratory tree. In the first two-thirds of the vessel, in the region of the small intestine, a large cross vessel connects the anterior and the middle part of the vessel (Plate 6). Posteriorly, in the region of the large intestine, the main vessel becomes smaller, lies close to the canal, and terminates in lacunar spaces.

As in other holothurians belonging to the same order there are five radial perihæmal canals. In the diagram of the cross section of the body wall through the radial nerve cord and its accompanying vessels (Plate 9, fig. 35) the axial space between the outer wall of the radial water canal and the deep oral system of the radial nerve cord is the perihæmal sinus.

The gonads are directly supplied with blood from the pharyngeal blood ring (Plate 6).

Externally the blood vessels appear like true vessels, but histological examination shows that they are filled with cells and strands of connective tissue with the blood corpuscles between these strands (Plate 4, fig. 2). Even those that have a lumen do not appear like true blood vessels. The wall is a structure not as sharply defined as that of the blood vessels of vertebrates. It is made up of loose strands of connective tis-

sue mixed with a few cells and surrounded by an outer, indistinct, muscular layer. The fluid within the system is a colorless plasma with occasional wandering cells.

THE REPRODUCTIVE SYSTEM

The reproductive system of *S. choronotus* consists of a genital duct and tufts of genital tubules. The duct opens to the exterior in the mid-dorsal interradius about 10 millimeters behind the tentacles (Plate 1, fig. 1). The duct that runs ventro-posteriorly from this genital opening into the dorsal mesentery up to the pharyngeal blood ring is not very distinct. It is usually much dilated and hidden in the mesentery. Beyond the pharyngeal blood ring, however, the duct can be seen distinctly within the dorsal mesentery. About 5 millimeters posterior to the blood ring it divides into two bundles of genital tubules (Plate 6). These bundles of genital tubules are located on both sides of the dorsal mesentery, and each of them consists of four to seven, or even more, genital tubules hanging freely in the body cavity. They branch dichotomously into smaller branches and extend sometimes as far as the posterior part of the body cavity. Some of them may interlock with the smaller branches of the respiratory trees.

The wall of the gonads (testis or ovary) is made up of the usual peritoneal epithelium, a thin layer of circular muscle fibers, a layer of connective tissue with some lacunar spaces, and an inner germinal epithelium which is scattered in more or less irregular masses (Plate 4, figs. 1, 4). The germinal epithelium produces either sperms or eggs, as the case may be, the sexes being separate. Externally there is not much difference between the testis and the ovary. The eggs are typical echinoderm eggs. The mature unfertilized egg is from 55 to 112 microns long; it has a large clear nucleus and one prominent nucleolus (Plate 4, fig. 1). The immature eggs within the ovary are suspended by a delicate membrane from the germinal epithelium and are really within the egg follicles. The sperm cell is minute, approximately 1 micron in length. The mature sperm when seen under an oil-immersion lens appears as a pear-shaped body with a short tail, free within the lumen of the tubule. Younger male germ cells in early stages of spermatogenesis are usually found grouped together within the germinal epithelium (Plate 4, fig. 4).

THE NERVOUS SYSTEM

The nervous system of *S. chloronotus* is divided into two parts, the ectoneural ventral system and the deep oral system. The first consists of the circumoral nerve ring with the tentacular and buccopharyngeal nerves and the five radial, longitudinal, nerve cords with branches to the tube feet, the papillæ, and the integument. The latter is composed of a double radial nerve band closely applied to the radial nerve cord of the ectoneural ventral system (Plate 7, fig. 1; Plate 9).

The circumoral ring is flattened and is fairly prominent. It is about 1 millimeter wide and 0.2 millimeter thick. It lies beneath a connective-tissue layer immediately internal to the bases of the tentacles anterior to the calcareous ring (Plate 5, fig. 1). It gives rise to the tentacular nerves, which run forward on the inner face of the stalk of each tentacle, forming a wide sheet. It is thickest near its base and smallest at its distal part. This gives off fibers directly to the sensory part of the tentacles. Some isolated fibers find their way to individual neuroepithelial cells. The buccopharyngeal nerves extend radially inward from the nerve ring to the buccal sphincter muscles, then turn backward along the pharynx. They innervate the muscles and the epithelium of the pharynx.

The radial nerves form a thick outer cord as a part of the ectoneural ventral system, and two thin closely attached inner bands as a part of the deep oral system. The thick outer portion is considered to be the sensory center and the thin inner portion a motor center (Herouard, 1887). The inner bands anteriorly divide and subdivide into branches and innervate the neighboring longitudinal and circular muscles and also send nerves to the tube feet. The outer cord runs posteriorly as far as the cloacal opening and gives off branches to the tube feet, as pedal nerves, and also to the papillæ and the integument. The side branches of the radial nerve cord are of two groups. Those of one group run to the interradius where they constitute a true plexus, homologous with the superficial plexus of the test of the sea urchin (Herouard, 1887); those of the other group run along the ambulacral tube and innervate the walls of the tube feet and the papillæ (Plate 9, fig. 35). Each radial nerve is accompanied by a tubelike cavity, closed at both ends, called the hyponeural canal on its inner side and the epineural canal on its outer side (Plate 7, fig. 1; Plate 9). The latter extends

to the upper and the inner side of the circumoral nerve ring and on the inner side of the tentacular nerve.

THE SENSE ORGANS

The tentacles constitute the principal organs of touch, although the tube feet may also function as such (Plate 1, figs. 1, 2). Sensory nerve cells that are the immediate seat of tactile sensations are scattered all over the epithelial covering of the body. They are most abundant in the extremities of the tentacular branches (Plate 7, fig. 2).

PARASITES

Stichopus chloronotus at Puerto Galera often harbors a commensal fish, *Fierasfer homei* Richardson (Plate 1, fig. 3).¹ A similar fish inhabits the body cavity of *Holothuria mammifera* (Kent, 1893). The colored drawing given by Kent (his fig. 10, Chromo XII) is similar in external appearance to the commensal fish that seeks shelter in *S. chloronotus*, but of a different species. Fishes of the same genus have also been reported by Kent (1893) to live in the mantel cavity of *Meleagrina margarilifera*.

Stichopus chloronotus shelters other commensal animals. Among these is an annelid, a species of *Gastrolepidia*, whose color closely approximates that of its host and whose form enables it to retain its position even against a considerable effort to detach it (Clark, 1921). Another one, reported by Kent (1893), is a species of flat large-scaled worm, closely allied to *Polynoë setosa*, which clings closely to the integument in the interspaces between the projecting papillæ; one of the Puerto Galera specimens were found with similar animals. In life it can be scarcely detected owing to its perfectly harmonizing colors. The Puerto Galera and Lingayen Gulf specimens are not entirely free from such or similar parasites.

The intestines of almost all the specimens of *S. chloronotus* collected in 1926 show intestinal appendages in the form of long tubules (Plate 8, fig. 7). The tubules are double walled, and within the inner tube are many microscopic, embryolike organisms that apparently are parasites (Plate 8, fig. 8). An examination under an oil-immersion lens shows that these organisms are made up of amœboid cells with clear cytoplasm and reticular nuclei. The greatest portion of this organism is made up of granules which do not show any indication of being

¹ Identified by Mr. H. R. Montalban, of the Bureau of Science, Manila.

cells (Plate 8, fig. 8). The nature of these intestinal appendages needs further investigation.

J. Müller (1852) mentioned the development of testaceous gasteropods within a peculiar tube attached to the intestinal vessels of *Synapta digitata*. Baur (1861), on the same problem, demonstrated that the molluscigerous sac is an organism complete in itself that is called *Helicosyrinx parasitica*. The ones found in *S. chloronotus* are different from those described by Müller and Baur, and they are reported here simply because they constitute a puzzling problem for the morphology worker, especially in the beginning stages of the work.

DISCUSSION

Although *Stichopus chloronotus* has been studied by several workers their descriptions are brief and incomplete. They all agree on the general body form, the arrangement of the papillæ and the tube feet, the number of tentacles, the Polian vesicles, the stone canal, and the presence of certain body spicules. They differ, however, in the description of the body color. The differences in the colors of the animals described may be due to differences in the localities from which they were obtained and to the difference in the condition of the animals when described. Those obtained from different localities vary from each other, showing that different environmental condition affect the color of the animal. *Stichopus chloronotus* described by most of the workers were preserved specimens whose color must have changed as a result of preservation. Théel (1886) said that his specimens were contracted and wrinkled showing that they had been preserved for sometime. Fisher (1907) also must have described preserved specimens as the color he gave is the same as that of Théel, which is olive-brown, the actual color usually associated with the specimens when preserved in 10 per cent formalin.

The list of types of spicules as found and described by most of the authors is not complete. Although Théel and Fisher mentioned many types of spicules, they left many unmentioned and undescribed. The large quadrangular supporting rods (Plate 2, figs. 1-3) and large tables (Plate 2, figs. 5-9) found in the wall of the stalk of the tentacles were never mentioned. It is likely that these types of spicules were overlooked by them, or possibly they are peculiar to the Puerto Galera form. Previous workers made no mention of the spicules of the internal

organs, like the rod-shaped deposits in the walls of the alimentary canal, the water-circular canal, the Polian vesicles, the suspensors of the pharynx, etc.; the X-shaped and $><$ -shaped spicules in the walls of the alimentary canal, and the rosette-shaped spicules in the main respiratory tree trunks. All types of spicules previously reported are found in Puerto Galera specimens. Some of the spicules of *S. chloronotus* are similar to those of *S. tropicalis* Fisher, *S. variegatus* Semper, and others of the same genus. The C-shaped spicules (Plate 2, figs. 30, 31), the simple tables (Plate 2, figs. 22-29), and the spinous supporting rods of the pedicel (Plate 3, figs. 16-19) appear to be present in most of them.

It is probable that some of the spicules reported here for the first time are absent in the specimens described by Théel (1886), Fisher (1907), and several others. Fisher reported that the rosettes, which Théel found in his specimens, were not present in the Hawaiian form. The spicules in the tentacles of *S. chloronotus* may be easily overlooked in preserved specimens in which the tentacles are usually retracted inside the buccal cavity. In such specimens the tentacles cannot be very well exposed for spicule examination. The spicules in the form of large tables (Plate 2, figs. 5-9), which are located in the proximal part of the stalks of the tentacles, would certainly be overlooked in the contracted form. The large quadrangular supporting rods, however, cannot be missed if the tentacles are examined microscopically, because they lie just posterior to the expanded part of the tentacle.

The length of time the specimens are preserved in formalin must also be taken into consideration. The slight acidity of formalin, which is usually used for preservation, is high enough to dissolve many spicules. The longer they are preserved the fewer spicules can be found. One specimen of *S. chloronotus*, collected at Puerto Galera in 1912, was found to possess all the different organs with the exception of the calcareous deposits. Not a single spicule was left. The color of the body became entirely bleached.

With the exception of some slight variations, the general plan of the different systems of the body is similar to those of the holothurians belonging to the same order.

Semper (1868) noted some muscular tubules traversing the connective-tissue layer of the body wall. He stated that these tubules are connected to the radial canal. Gerould (1896) found that these muscular tubules in *Caudina arenata* are direct-

ly continuous with the transverse or circular muscles of the body wall. In *S. chloronotus* these muscular tubules appear to be outgrowths of the circular muscles similar to *Caudina arenata* as found by Gerould. However, the muscular tubules in the species under discussion are found only in the dorsal ambulacral regions. They resemble regular vessels with some cells within the lumen. If these muscular tubules are true outer extensions of the circular muscles, it would be interesting to find out why the muscle fibers are arranged in the form of hollow cylinders with cells within the lumen. Gerould may be right in his explanation that these muscular tubules may be rudimentary ambulacral vessels whose central ends have lost their primitive connection with the radial canal and which become united secondarily to the transverse muscles of the body wall.

Gerould (1896) said that in *C. arenata* the longitudinal muscle fibers in the cloaca are entirely absent, while Clark (1907) stated that they are functionally replaced by about twenty isolated, irregularly arranged, small, longitudinal muscles lying outside the circular-muscle layer. In *S. chloronotus* both circular- and longitudinal-muscle layers are found. Contrary to Clark's findings, the longitudinal muscle is within the circular-muscle layer.

The arrangement of the muscle layers in the wall of the alimentary tube of *Caudina* is the same as in all Molpadiidæ and Cucumariidæ where the longitudinal-muscle fibers lie within the circular-muscle layer (Gerould, 1896). In *Holothuria* a similar arrangement of muscle layers is found in the regions of the pharynx and the stomach only. In the region of the small and the large intestine the longitudinal-muscle layer lies outside the layer of circular-muscle fibers (Hamann, 1884). In *S. chloronotus* the arrangement of the muscle layers agrees with that described by Gerould and others.

In *Cucumaria frondosa* the stomach lacks the inner connective-tissue layer (Gerould, 1896). In *C. cucumis* and *C. planci* the inner connective-tissue layer is lacking in the pharynx and insignificant in the wall of the stomach (Hamann, 1896). In *S. chloronotus*, however, the inner connective-tissue layer is present in both pharynx and stomach.

Semper (1868) found perforations at the tips of the lateral branches of the respiratory trees in *S. variegatus*. Sluiter (1887) and Hamann (1884) found similar structures in other holothurians. Kingsley (1881) and Gerould (1896) were unable to find in *Caudina* any perforation at the tips of the branches

of the respiratory trees. Repeated attempts failed to show any perforations at the tips of the respiratory branches in *S. chloronotus*.

Gerould (1896) found a single layer of muscle fibers in the muscular layer of the respiratory tree. He said that the muscle fibers run in all directions parallel to the surface. This was confirmed by Jourdan (1883) on *Cucumaria planici*. In *Holothuria tubulosa*, Semper (1886) and Hamann (1884) found two layers, corresponding to those of the intestine; namely, an inner layer of longitudinal fibers and an outer one of circular fibers. In *S. chloronotus* two layers of muscle fibers are found.

Jourdan (1883) did not recognize that the radial nerve is made up of an outer and an inner band. Hamann (1883-84) denied the existence of the connective-tissue partition between the two parts of the radial nerve cord. Semper (1868), Herouard (1887), Gerould (1896), Clark (1907), and Sedgwick (1909) recognized the inner and the outer part of the radial nerve cord. The inner part is said to be the deep oral system, and the outer is said to be a part of the ectoneural ventral system. Not all of them, however, recognized the fact that the inner part is double. Semper in his work on some holothurians showed the double parts of the deep oral system. Sedgwick (1909) mentioned it as being obscurely double. Two thin bands of the deep oral system are found in *S. chloronotus*, thus agreeing with the findings of Semper.

The five radial neural canals lying immediately below the five radial nerves were first found by Semper (1868). They were also noted by Théel (1882), Hamann (1884), Herouard (1889), Gerould (1896), Clark (1907), and others, so that the presence has been satisfactorily demonstrated throughout the whole group of Holothuroidea. These canals were called "pseudohæmal" by Hamann and "canal sous-nervien" by Herouard. Gerould proposed the term "hyponeural." Semper found that in Holothuriidæ these canals terminated blindly behind the nerve ring. The same is true in Cucumariidæ (Herouard) and in *Caudina* (Gerould). In *S. chloronotus* these hyponeural canals are also present and terminate blindly behind the nerve ring.

Similar canals are found on the outer part of the radial nerve cord and are called "epineural" by all investigators. They are generally regarded, however, as the result of an artificial separation of the external face of the radial nerve from the con-

nective-tissue layer of the body wall. Herouard (1889) and Gerould (1896), however, stated that these epineural canals are not the result of artificial breaks between the nervous and the connective tissue, but are normal cavities. In *S. chloronotus* these canals are also normal cavities as they are lined with regular epithelial cells. It is the opinion of Gerould that these canals are filled with a fluid similar to that found in the body cavity, which possibly protects the nervous system against injuries incident to violent contractions of the body wall.

SUMMARY

Stichopus chloronotus Brandt, collected from Puerto Galera, Mindoro, and Hundred Islands, Pangasinan Province, Luzon, Philippine Islands, ranges from 200 to 400 millimeters in length. It is deep blue or almost black, with the tips of the papillæ slightly lighter in color. Usually it has twenty tentacles, but sometimes it may have eighteen or nineteen. Its calcareous deposits are C-shaped, rosette, simple and complex tables, simple or branched rods, large quadrangular supporting rods, and supporting sieve plates.

The body wall consists of five parts; namely, the epidermis, the connective-tissue layer, the circular-muscle layer, the longitudinal radial muscles, and the lining epithelium. The body cavity is incompletely traversed by three mesenteries, which suspend the three coils of the alimentary canal.

The water-vascular system is made up of the circular canal, the five radial canals, the tentacular and ambulacral canals with their ampullæ, the stone canal, and the madreporite body. The madreporite opens by numerous pores to the body cavity, thus making the fluid contents of the water-vascular system and that of the body cavity identical with each other.

The alimentary canal is composed of mouth, pharynx, stomach, small and large intestines, cloaca, and anus. The stomach, the small intestine, and the large intestine are not distinctly marked off from each other. Histologically, the wall of the alimentary canal consists of five layers, as follows: An outer layer of epithelium, a thin outer layer of connective tissue, a muscular layer composed of circular- and longitudinal-muscle fibers, a thick inner layer of connective tissue, and a lining epithelium.

The respiratory system is composed of two respiratory trees, which arise from a short common trunk on the anterior dorsal part of the cloaca. The left one is in close relationship with the

blood plexus of the dorsal, intestinal, hæmal vessel. The respiratory tree is an outgrowth of the intestine, and it is histologically similar to it.

The principal parts of the hæmal, or blood, system are the pharyngeal blood ring, the dorsal and ventral intestinal vessels, the radial perihæmal canals, and the lacunar spaces. The pharyngeal blood ring is posterior to the water circular canal. The two intestinal vessels are distinct from each other and are connected with the lacunar spaces of the alimentary canal by rete mirabile.

The reproductive system consists of a genital duct and tufts of genital tubules. The duct opens to the exterior in the mid-dorsal interradius about 10 millimeters behind the tentacles. The wall of the gonads is made up of peritoneal epithelium, a thin layer of circular-muscle fiber, a layer of connective tissue, and an inner germinal epithelium. The sexes are always separate.

The nervous system is divided into the ectoneural ventral system and the deep oral system. The first consists of a circumoral nerve ring, which gives rise to the tentacular and buccopharyngeal nerves, and to five radial nerve cords with branches to the tube feet, the papillæ, and the integument. The second is composed of double radial nerve bands closely applied to the radial nerve cord of the ectoneural ventral system. The principal sense organs are the tentacles, although some sensory nerve cells are found scattered all over the covering epithelium of the body.

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ILLUSTRATIONS

PLATE 1

FIG. 1. *Stichopus chloronotus* Brandt, dorsolateral view.

go, genital opening.

t, tentacles.

tf, tube feet.

dlp, dorsolateral papillæ.

vlp, ventrolateral papillæ.

dia, dorsal interambulacrum.

lia, lateral interambulacrum.

an, anus.

2. *Stichopus chloronotus* Brandt, anteroventral view.

t, tentacle.

op, oral papilla.

st, stalk of tentacle.

m, mouth.

p, papilla.

tf, tube feet.

via, ventral interambulacrum.

3. *Fierasfer homei* Richardson, lateral view. This fish is commensal with *S. chloronotus*.

Plates 2 to 11 illustrate the internal anatomy of *Stichopus chloronotus* Brandt.

PLATE 2

FIG. 1. Large straight quadrangular rod; actual length, 796 microns.

2. Large curved quadrangular rod; actual length, 647 microns.

3. Small curved quadrangular rod; actual length, 185 microns.

4. Small straight quadrangular rod; actual length, 92 microns.

5. Side view of large table with four pillars; actual height, 74 microns.

6. Ventrolateral view of large table with six pillars; actual height, 111 microns.

7. Dorsolateral view of large table with six pillars; actual height, 56 microns.

8. Dorsal view of large table with six pillars, actual diameter of edge, 56 microns.

9. Lateral view of large table with six pillars; actual height, 148 microns.

10. Small sievelike rosette; largest diameter, 33 microns.

11. Large sievelike rosette; largest diameter, 44 microns.

12. Simple incomplete rosette; actual length, 130 microns.

13. Rosettelike spicule; actual width, 37 microns.

14. Simple incomplete rosette; actual size, 37 microns.
15. Simple incomplete rosette; actual length, 37 microns.
16. Small sievelike rosette; largest diameter, 37 microns.
17. Simple rosette; actual length, 37 microns.
18. Simple rosette; actual length, 37 microns.
19. Rodlike spicule; actual length, 93 microns.
20. Simple incomplete rosette; actual length, 56 microns.
21. Wrenchlike spicule; actual length, 93 microns.
22. Lateral view of a simple table found in the body wall; actual height, 37 microns.
23. Modified simple table from the same region as fig. 22.
24. Same as fig. 23, seen from a different angle; actual height, 37 microns.
25. Lateral view of a simple table with short pillars; actual height of pillars, 20 microns.
26. Dorsal view of a simple table (compare fig. 22); actual width, 30 microns.
27. Base of a simple table (compare fig. 23); actual width, 37 microns.
28. More complex form of a base of a simple table; actual width, 37 microns.
29. Base of a simpler form of a simple table; actual width, 37 microns.
30. C-shaped spicule of body wall; actual length of its longest axis, 37 microns.
31. Variation of C-shaped spicule of body wall.

PLATE 3

- FIG. 1. Supporting sieve plate at the margin of the tips of tube feet; actual measurement in its longest diameter, 252 microns.
2. Supporting sieve plate at the central portion of the tips of tube feet; actual diameter, 222 microns.
 3. Branched spicule from the gonad; actual length, 92 microns.
 4. X-shaped spicule from the gonad; actual height, 111 microns.
 5. Rodlike spicule from the gonad; actual length, 111 microns.
 6. Small rod-shaped spicule from the suspensor of the pharynx; actual length, 37 microns.
 7. Variation of fig. 6; actual length, 63 microns.
 8. Variation of fig. 6; actual length, 92 microns.
 9. Variation of fig. 6; actual length, 92 microns.
 10. Rod-shaped spicule from the walls of the alimentary canal; actual length, 130 microns.
 11. Branched spicule from the walls of the alimentary canal; actual length, 130 microns.
 12. Rod-shaped spicule from the walls of the alimentary canal; actual length, 111 microns.
 13. X-shaped spicule from the walls of the alimentary canal; actual height, 99 microns.
 14. Y-shaped spicule from the walls of the alimentary canal; actual length, 130 microns.

15. Forked spicule from the walls of the alimentary canal; actual length, 148 microns.
16. Spinous supporting rod of tube feet with sieve-plated ramifications at the middle; actual length, 333 microns.
17. Variation of fig. 16; actual length, 333 microns.
18. Variation of fig. 16, with simple branch at the middle; actual length, 300 microns.
19. Variation of fig. 16, with spinous processes at the middle; actual length, 350 microns.
20. Portion of calcareous ring showing a radial piece and interradial pieces; actual height of the radial piece, 10 millimeters.
21. Interradial piece of calcareous ring; actual height, 5 millimeters.

PLATE 4

FIG. 1. Small portion of a cross section of an ovary.

eth, external epithelium.

cm, circular muscle layer.

ct, connective tissue.

ge, germinal epithelium.

fc, follicular cell.

2. Cross section of a blood vessel.

eth, external epithelium.

m, indistinct muscle layer.

cs, connective-tissue strand.

sc, spheruliferous corpuscle.

bc, blood cell.

3. Cross section of a small part of the hæmal-respiratory-tree plexus showing the intimate relationship between the vessel and the minute branches of the tree.

bv, blood vessel.

wc, wandering cell.

sp, respiratory-tree branch.

4. Cross section of testis.

eth, external epithelium.

cm, circular muscle.

gc, germ cells in their early stages of spermatogenesis.

ct, connective tissue.

ge, germinal epithelium.

sc, spermatozoön.

5. Small complex rosettelike spicule from the trunk of the respiratory tree; actual diameter, 110 microns.

6. Large complex rosettelike spicule from the trunk of the respiratory tree; actual diameter, 190 microns.

PLATE 5

FIG. 1. Inner surface of the calcareous ring, circular water canal, and its branches.

t, tentacle.

st, stalk of tentacle.

tn, tentacular nerve.

nr, nerve ring.
cr, calcareous ring.
mp, madreporite.
sc, stone canal.
pv, Polian vesicle.
rwc, radial water canal.
twc, tentacular water canal.
ta, tentacular ampulla.
mrw, main radial water canal.
cwc, circular water canal.
pr, pharyngeal blood ring.

2. Small section of the pharynx and the pharyngeal blood ring.

ie, internal epithelium.
sp, suspensor of the pharynx.
s, spicule.
ps, peripharyngeal sinus.
pr, pharyngeal blood ring.
pw, communication between pharyngeal blood ring and water canal.
bc, blood cells.
wc, circular water canal.
pvo, opening of Polian vesicle into circular water canal.
pv, Polian vesicle.
p, wall of pharynx.

PLATE 6

A specimen opened along the dorsal interambulacrum showing the general anatomy.

t, tentacle.
ta, tentacular ampulla.
cr, calcareous ring.
m, madreporite body.
s, suspensors.
sc, stone canal.
cw, circular water canal.
pr, pharyngeal blood ring.
pv, Polian vesicle.
gd, genital duct.
bw, body wall.
g, gonad.
rt, right respiratory tree.
rw, radial water canal.
lm, longitudinal muscle.
cm, circular muscle.
ct, common respiratory-tree trunk.
cl, cloaca.
p, papilla.
tf, tube feet.
lt, left respiratory tree.
rhp, respiratory-hæmal plexus.

cs, connective strands.
a, anus.
s, stomach.
si, small intestine.
li, large intestine.
dv, dorsal intestinal hæmal vessel.
vv, ventral intestinal hæmal vessel.
cv, cross-ventral intestinal hæmal vessel.

PLATE 7

FIG. 1. Cross section through the radial nerve cord and its accompanying vessels.

lm, longitudinal muscle.
rs, radial blood sinus, or perihæmal sinus.
rw, radial water canal.
hc, hyponeural canal.
rni, inner band of the deep oral system.
rno, outer nerve band of the deep oral system.
rne, radial nerve cord of the ectoneural ventral system.
ep, epineural canal.
ce, cœlomic epithelium.
ct, connective tissue.
lc, lacunar space.
cm, circular muscle layer.

2. Longitudinal section of a small portion of the tip of the tentacle.

e, epithelium composed of mostly supporting and sensory cells.
sc, spheruliferous corpuscle.
tc, part of the tentacular canal.
lm, longitudinal muscle.
ct, connective tissue.

3. Inner surface of one ambulacrum showing the longitudinal muscle and its relation to the radial piece of the calcareous ring.

rw, radial water canal.
tc, tentacular canal.
cr, calcareous ring.
lm, longitudinal muscle.
cm, circular muscle.
s, sphincter.

PLATE 8

FIG. 1. Fragment of the calcareous ring; actual length, 74 microns.

2. Variation of fig. 1; actual length, 150 microns.
3. Variation of fig. 1; actual length, 111 microns.
4. Variation of fig. 1; actual length, 37 microns.
5. Variation of fig. 1; actual length, 150 microns.
6. Variation of fig. 1; actual length, 150 microns.
7. Small portion of the large intestine, showing the intestinal appendages caused by a parasite.

iw, intestinal wall.
ia, intestinal appendage.
dv, dorsal intestinal hæmal vessel.

8. Longitudinal section of one mature intestinal appendage.
 - ot*, wall of outer tubule.
 - it*, wall of inner tubule.
 - eo*, embryolike organism.
 - gr*, granules.
 - ac*, amœboid cell.
 - wc*, wandering cell.
 - mc*, mesenchyme cell.
9. Oval corpuscle from the wall of the alimentary canal; actual length, 18.5 microns.
10. Variation of fig. 9, from the body wall; actual length, 30 microns.
11. Sensory cell from the tentacle; actual length, 18 microns.
12. Similar cell from the body wall; actual length, 18 microns.
13. Supporting cell from the tentacle; actual height, 25 microns.
14. Supporting cell from the body wall; actual height, 20 microns.
15. Spheruliferous corpuscle from the body wall; actual length in its longest axis, 22 microns.
16. Spheruliferous wandering cell from the body wall; actual length, 37 microns.
17. Blood corpuscle; actual diameter, 3 microns.
18. Variation of fig. 17; actual diameter, 3 microns.

PLATE 9

Diagram of the cross section of the body wall through the median ventral ambulacrum.

- cm*, circular muscle.
- lm*, longitudinal muscle.
- ep*, epineural canal.
- rw*, radial water canal.
- rni*, inner nerve band of the deep oral system.
- rno*, outer nerve band of the same system.
- rne*, radial nerve cord of the ectoneural ventral system.
- rs*, radial blood sinus.
- hc*, hyponeural canal.
- v*, valves of the opening of the radial water canal into an ambulacral canal.
- ac*, ambulacral canal.
- pn*, pedal nerve.
- ce*, cœlomic epithelium.
- pa*, pedal ampulla.
- ed*, epidermis.
- ct*, connective tissue.
- st*, simple table.
- sc*, spheruliferous corpuscle.
- sp*, C-shaped spicule.
- pm*, pedal longitudinal muscle.
- pc*, pedal canal.
- sp*, section of supporting sieve plate.
- sr*, section of supporting spinous rods.

PLATE 10

FIG. 1. Low-power drawing of the cross section of a respiratory-tree trunk.

- lb*, lateral branch.
- ee*, external epithelium.
- cm*, circular muscle.
- lm*, longitudinal muscle.
- ct*, inner connective-tissue layer.
- oct*, outer connective-tissue layer.
- ie*, internal epithelium.
- sp*, section of rosettelike spicule.

2. Small portion of a cross section of the intestine showing a bud of the intestinal appendage.

- lm*, longitudinal muscle.
- ia*, intestinal appendicular bud.
- ee*, external epithelium.
- oct*, outer connective-tissue layer.
- cm*, circular muscle.
- ict₁*, outer vacuolated and reticular part of the inner connective-tissue layer.
- ict₂*, inner homogeneous fibrous part of the inner connective-tissue layer.
- ie*, internal epithelium.
- sc*, spheruliferous corpuscle.

3. Cross section of the circular water canal.

- c*, cilia.
- ic*, internal epithelium.
- cm*, circular muscle.
- ct*, connective tissue.
- ce*, external epithelium.
- wc*, wandering cell.
- sp*, rodlike spicule.

4. Longitudinal section of a small part of the body wall through the longitudinal-muscle band.

- ce*, cœlomic epithelium.
- lm*, longitudinal muscle.
- cs*, connective-tissue strand.
- lc*, lacunar space.
- cm*, circular muscle.
- ct*, connective tissue.

5. Cross section of the stone canal.

- dm*, part of dorsal mesentery.
- ce₁*, columnar internal epithelium.
- ce₂*, cuboidal internal epithelium.
- c*, cilia.
- cb*, calcareous bodies.
- ct*, connective tissue.

6. Small portion of a cross section of the cloaca.

- cs*, connective-tissue strand.
- ee*, external epithelium.

oct, outer connective-tissue layer.
cm, circular muscle.
lm, longitudinal muscle.
ct, inner connective-tissue layer.
ie, internal epithelium.
sc, spheruliferous corpuscle.
cc, connective-tissue cell.

PLATE 11

Longitudinal section of madreporé body.

ch, chamber of the madreporé body.
c, cilia.
ee, external epithelium.
p, pore.
stc, stone canal.
dm, dorsal mesentery.
cb, calcareous bodies
ct, connective tissue.

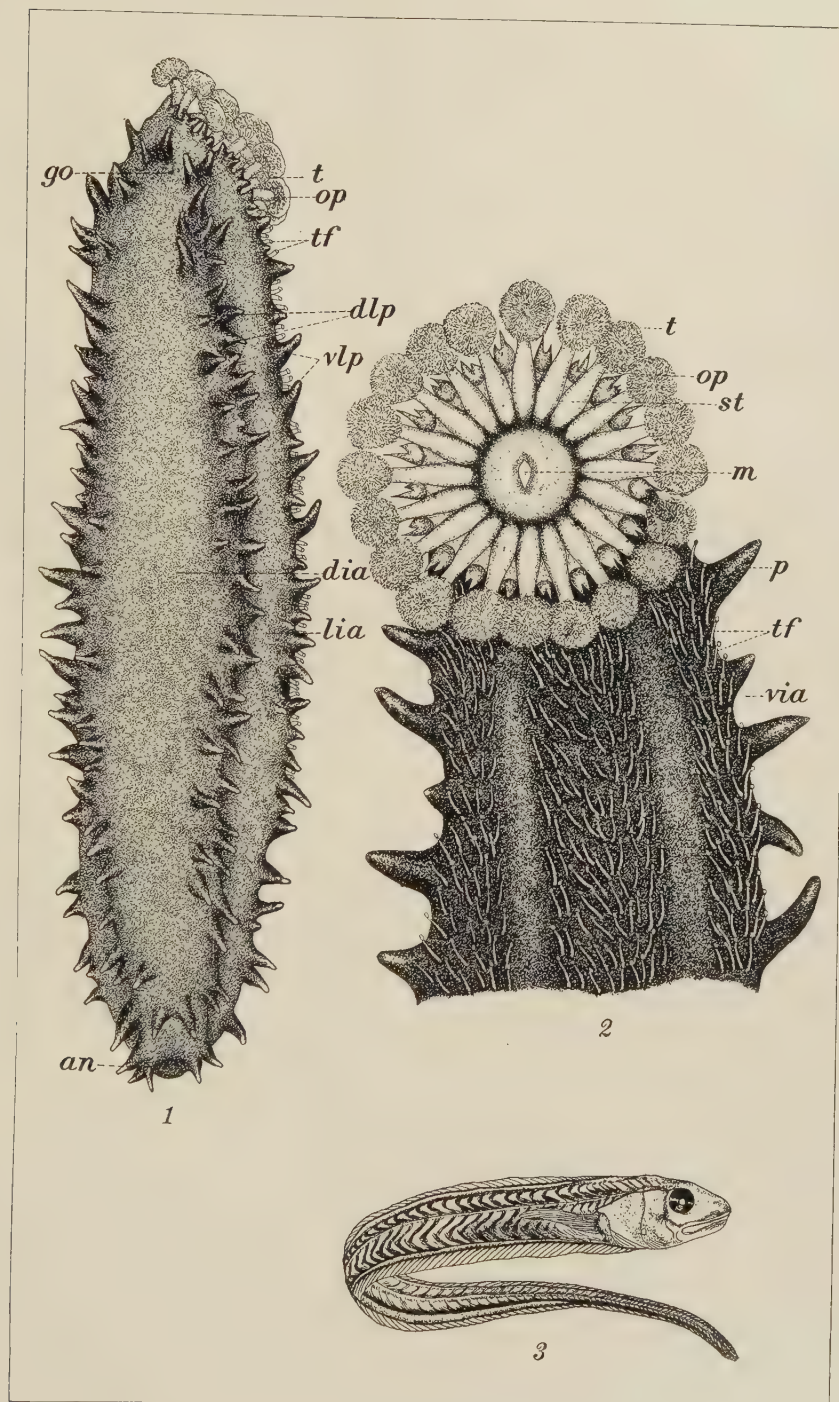


PLATE 1.

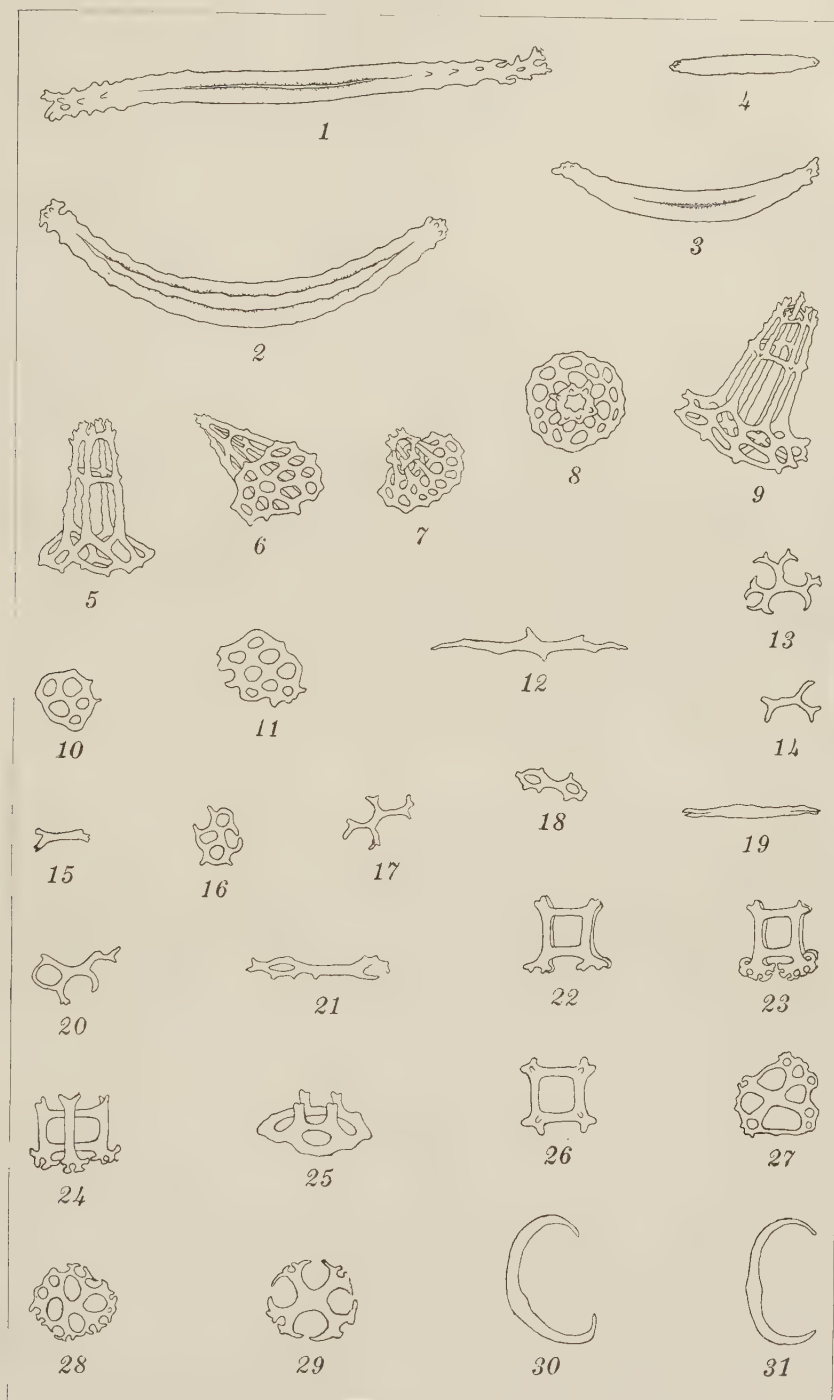


PLATE 2.

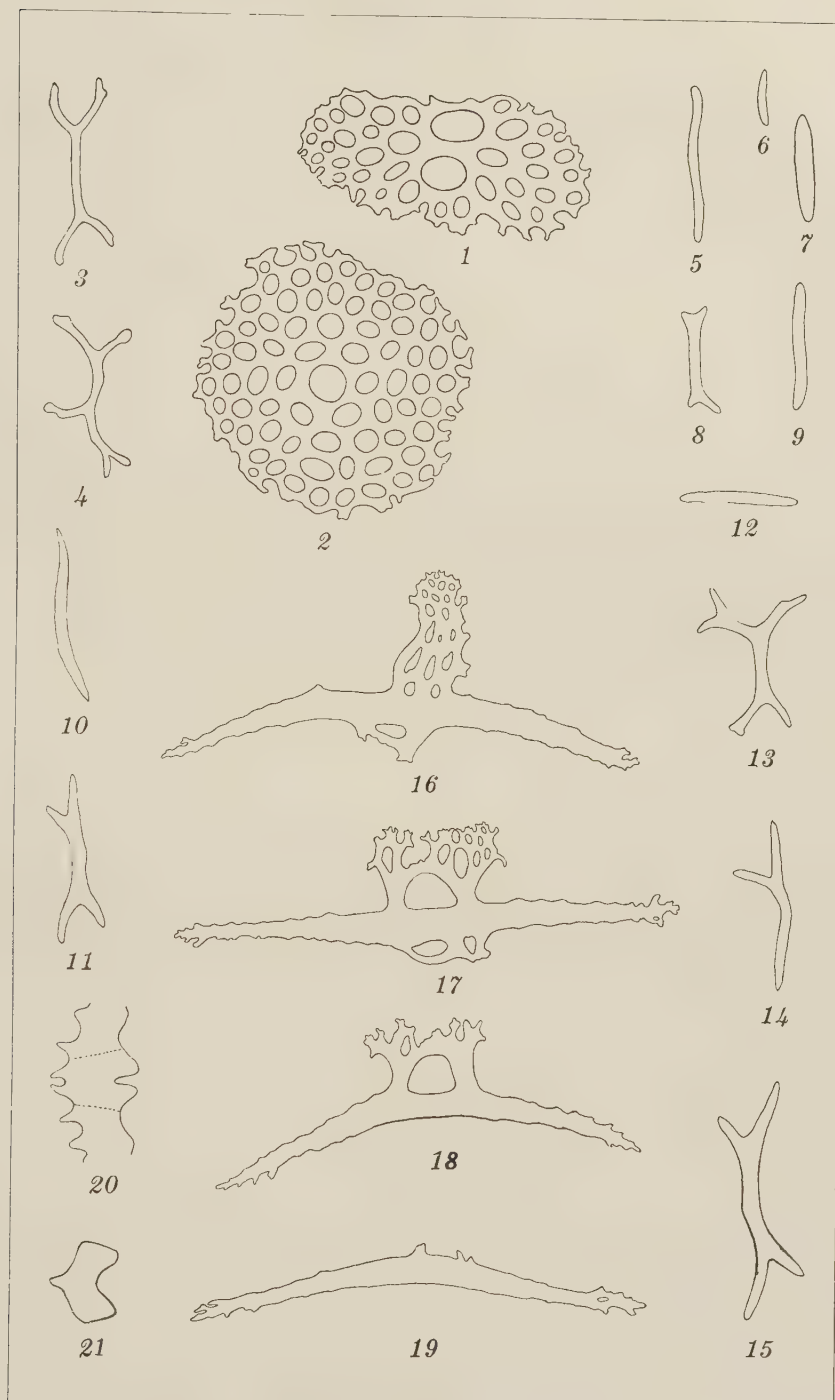
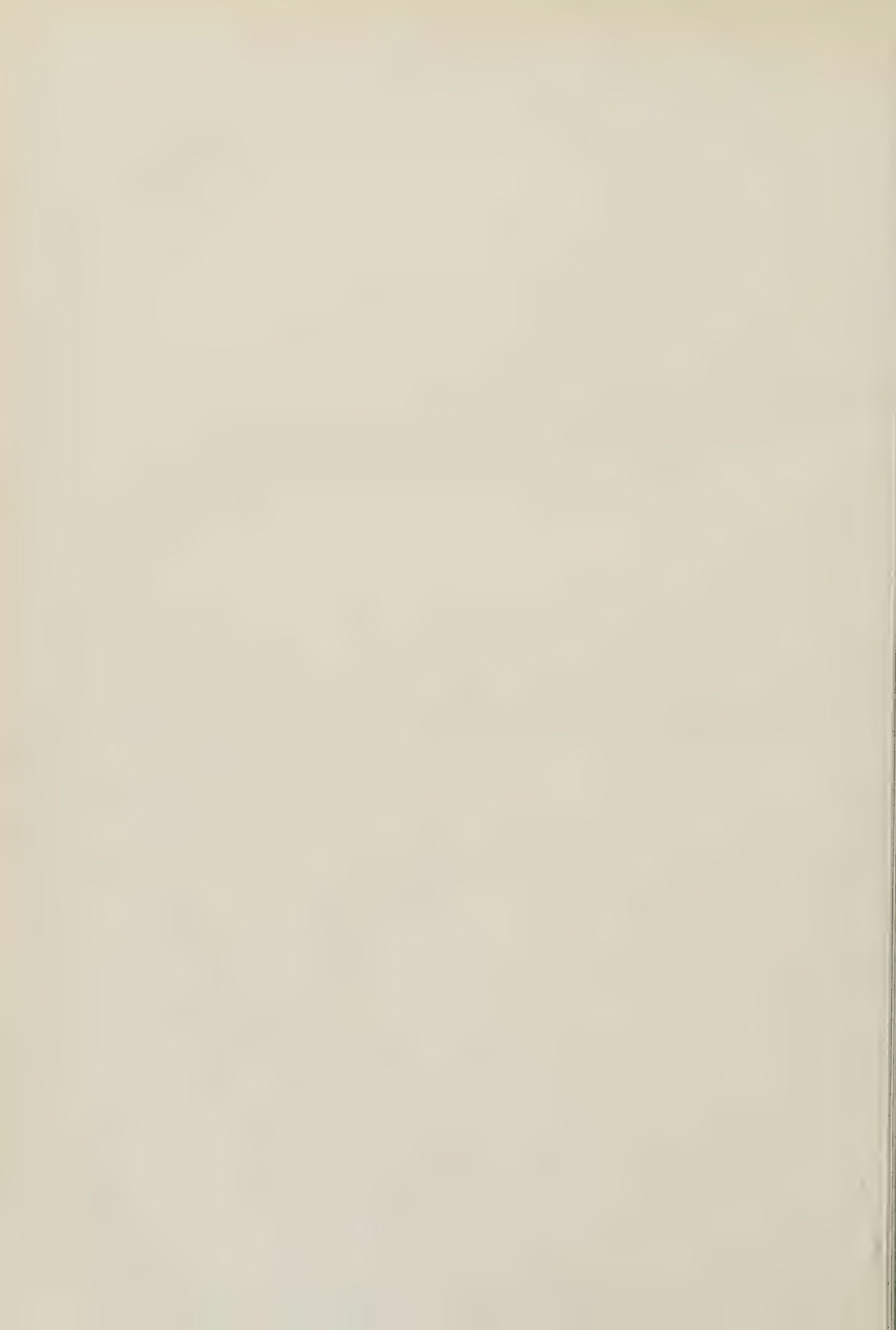


PLATE 3.



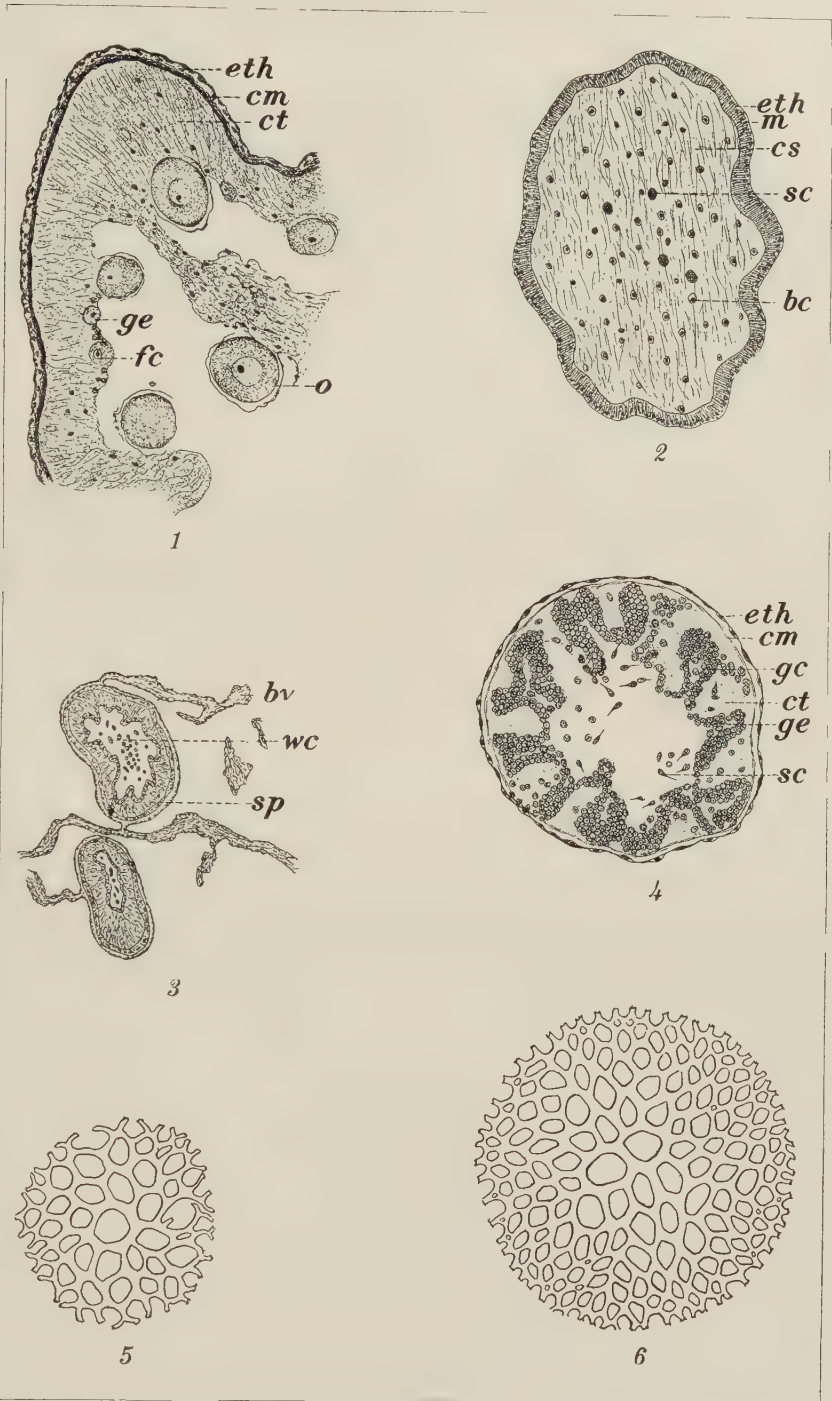


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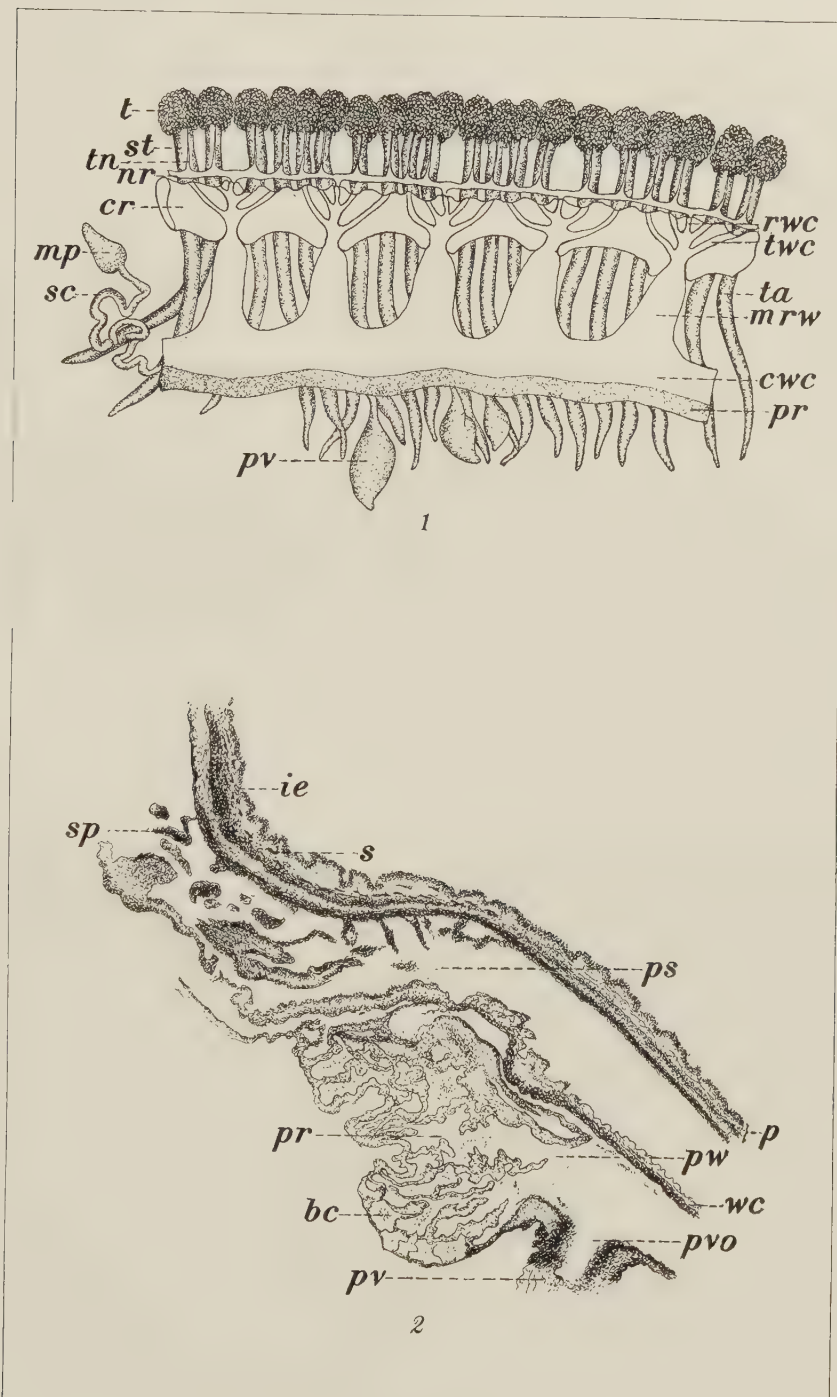


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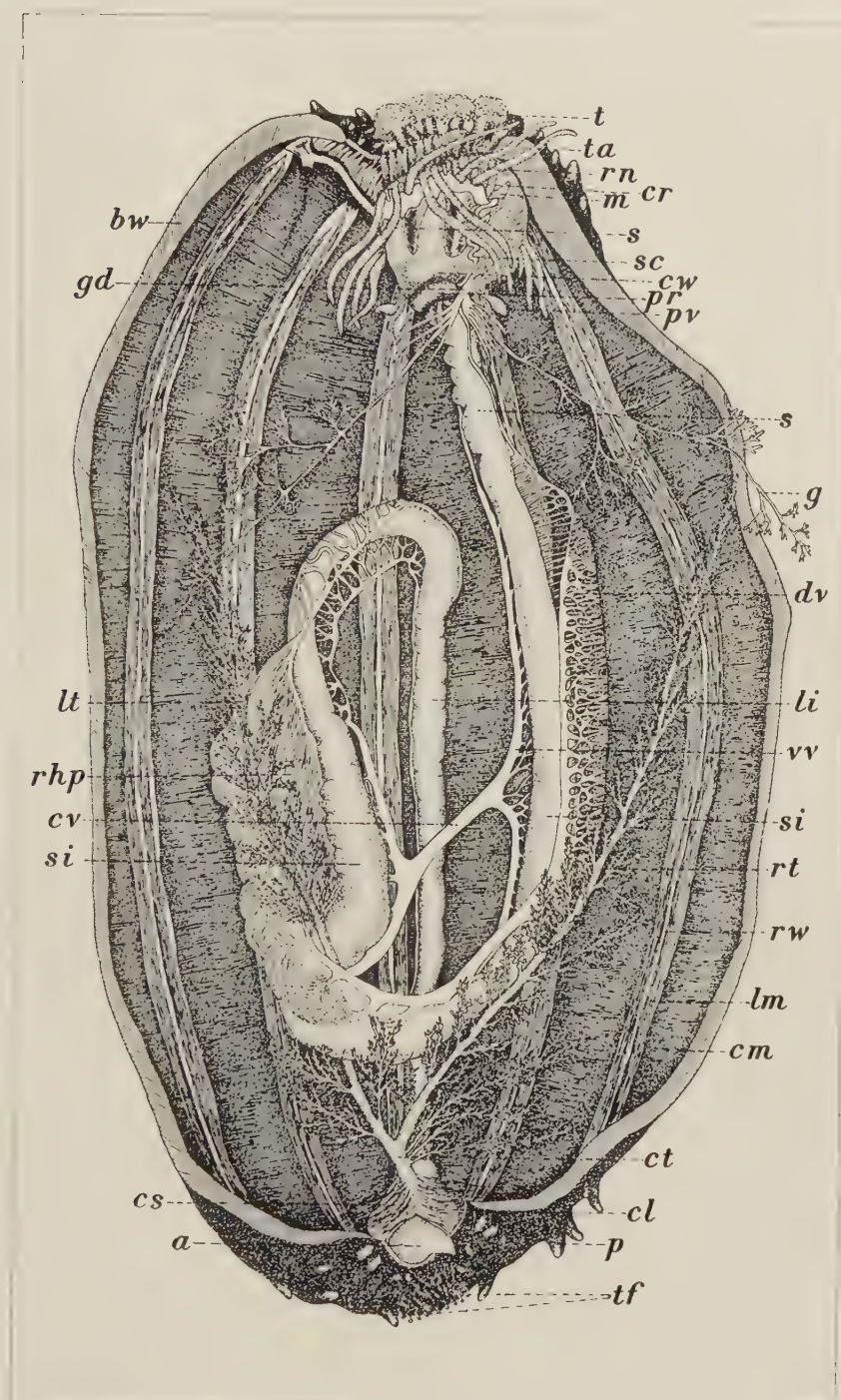


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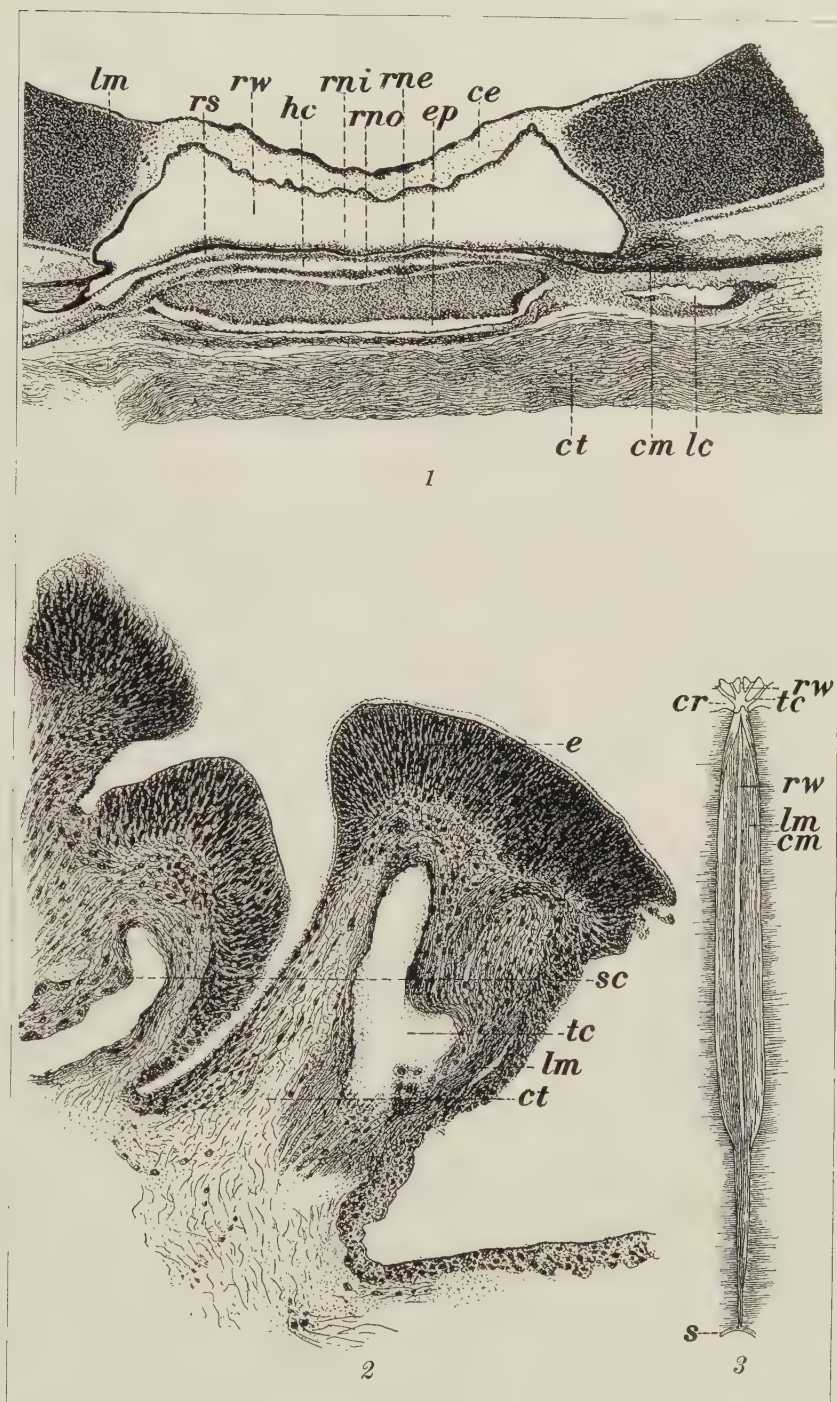


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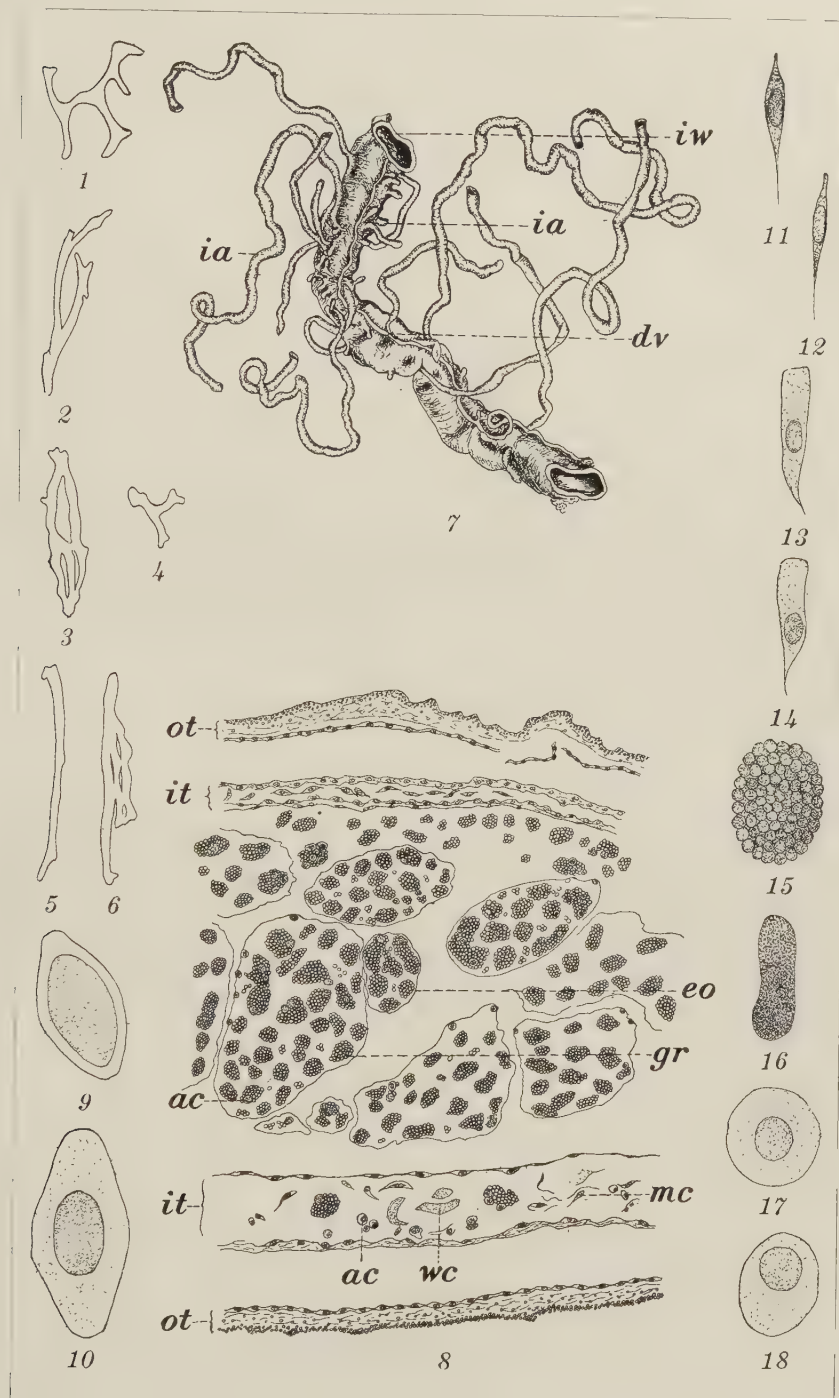


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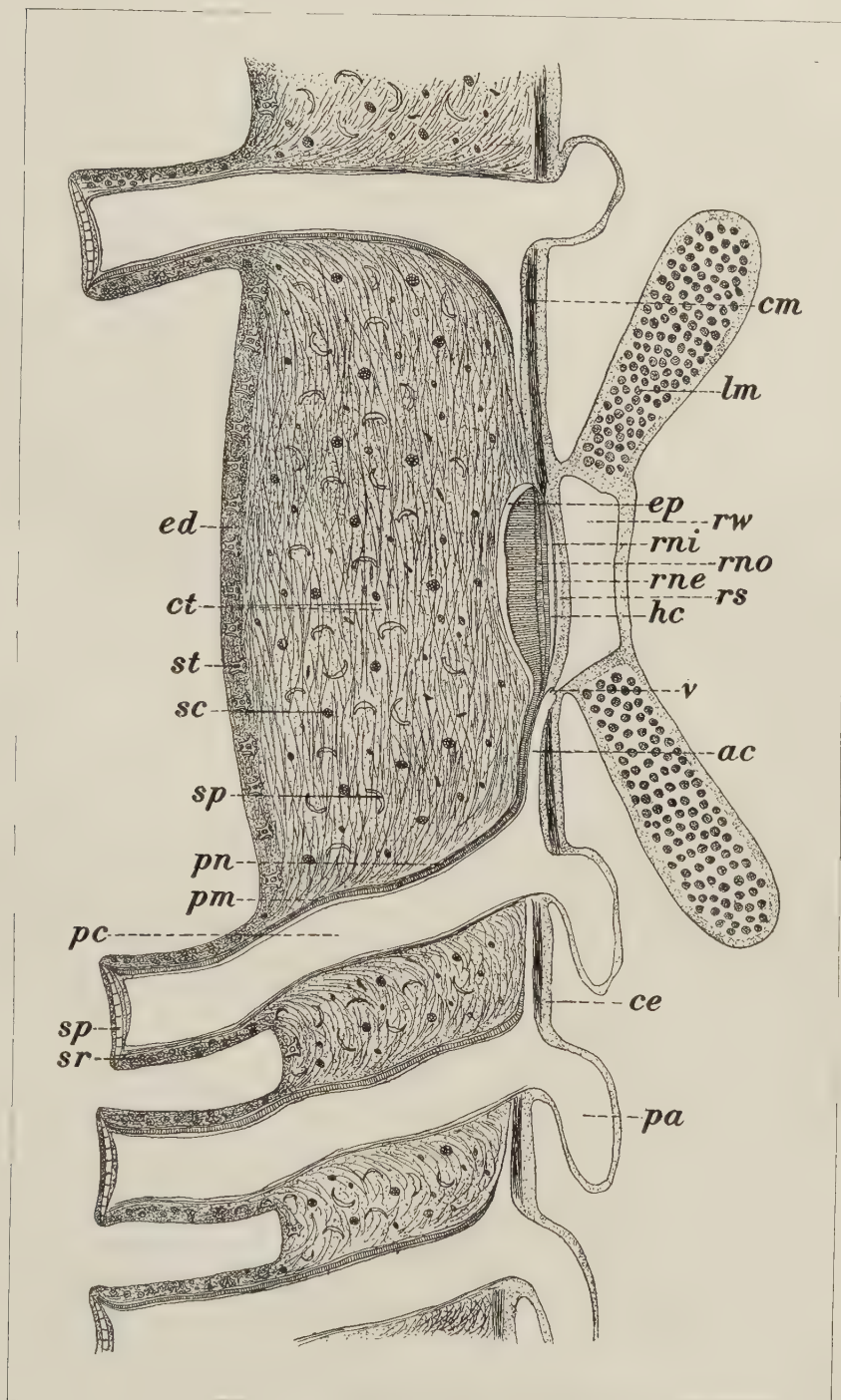


PLATE 9.

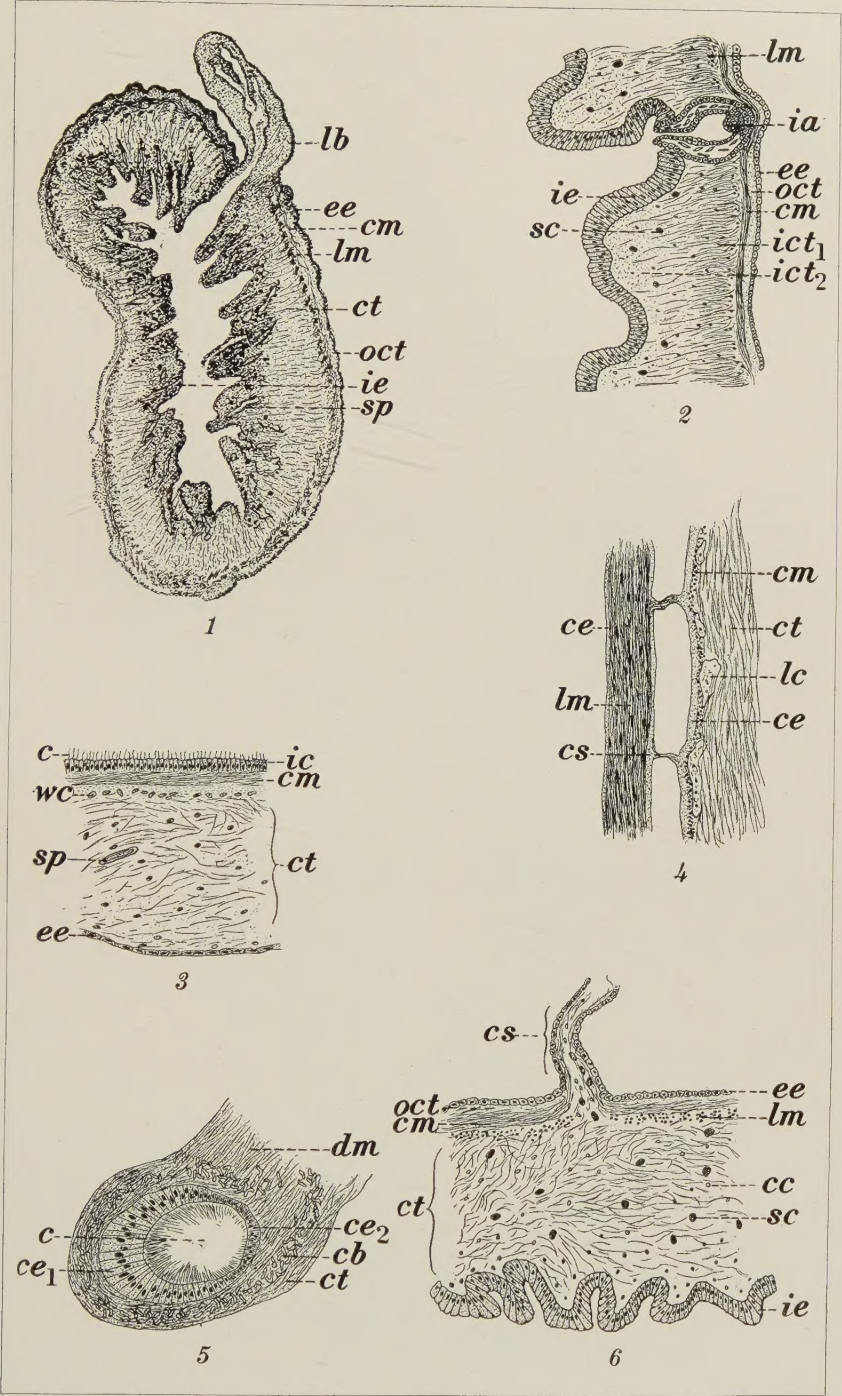


PLATE 10.

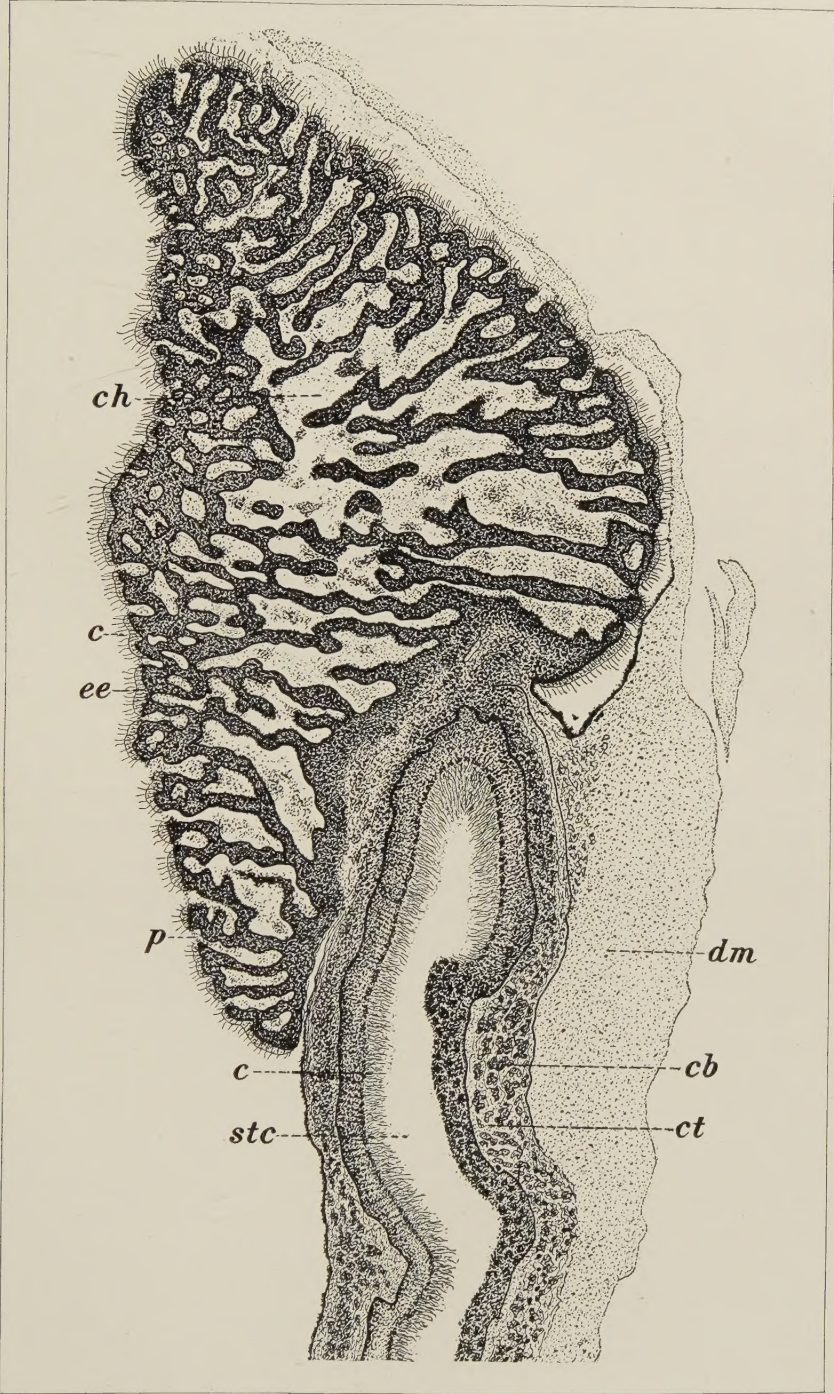


PLATE 11.

